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National Institute of Neurological
and Communicative Disorders
and Stroke

Intramural Research

Annual Report
Fiscal Year 1985

U.S. DEPARTMENT
OF HEALTH
AND HUMAN SERVICES
Public Health Service
National Institutes of Health

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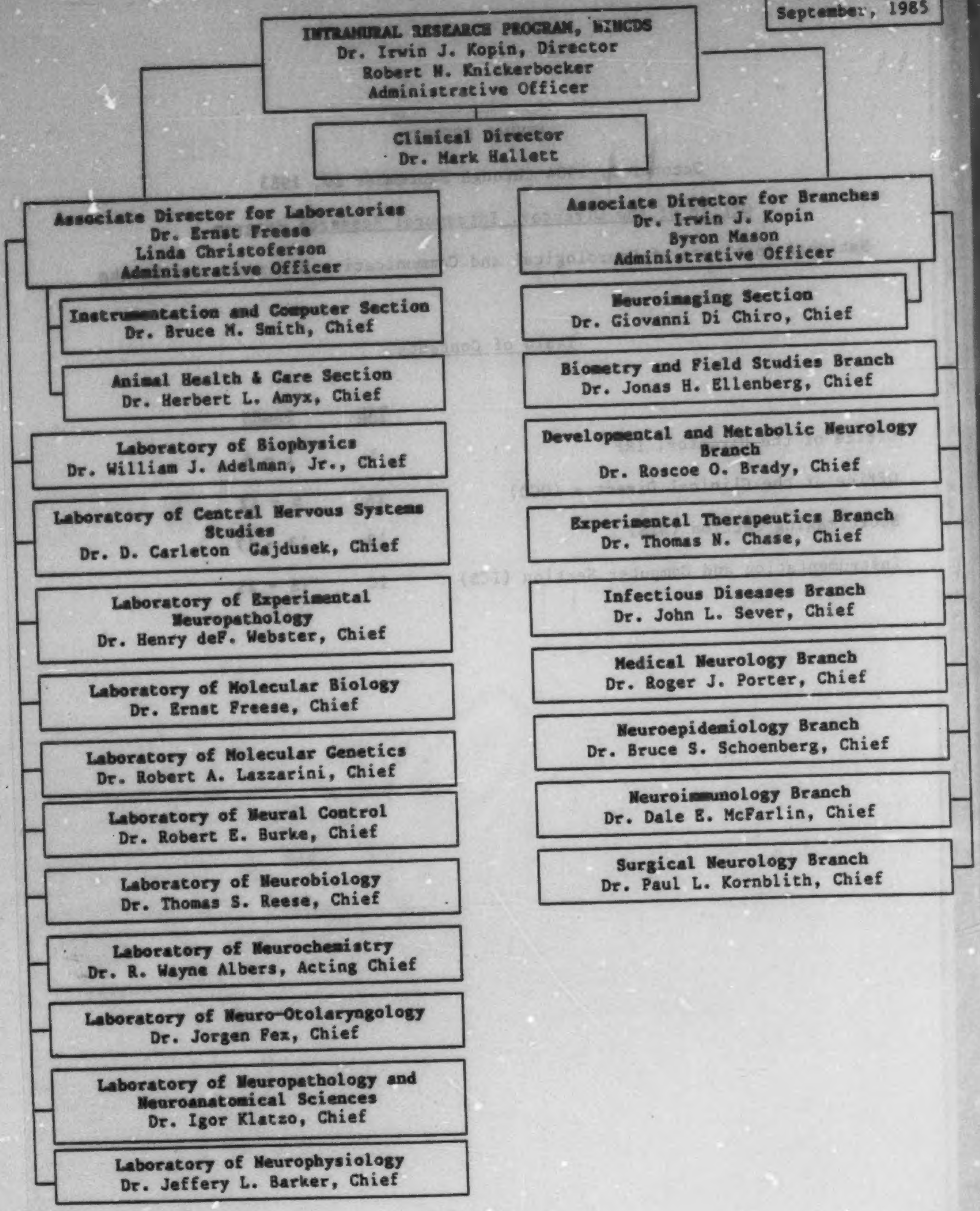
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Annual Report of the Scientific Director

National Institute of Neurological and Communicative Disorders and Stroke

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Irwin J. Kopin, M.D., Scientific Director

The Intramural Research Program conducts investigations by direct operations of laboratories and clinics mainly at the NIH complex in Bethesda. Portions of the research are also performed away from Bethesda, at Fort Detrick in Frederick, Maryland, and at the Marine Biological Laboratory in Woods Hole, Massachusetts. In these facilities, Federal Government scientists, their support staff, and guest research workers continue to discover and produce new knowledge that contributes to our ability to prevent, ameliorate or cure neurological or communicative diseases. The research efforts range from studies about chemical interactions of molecules to innovative therapeutic interventions with new drugs in patients. These studies contribute significantly to the explosive growth of new knowledge in the neurosciences and greater understanding of diseases of the nervous system. The research projects, which are investigator-initiated, all relate to the main mission of the Institute and the NIH: the advancement of biomedical knowledge for the ultimate prevention or alleviation of human suffering from disease or injury.

During the last year, the Intramural Research Program remained relatively stable with regard to its leadership, the recently appointed Director, IRP completing his second year and Clinical Director his first year in their respective positions. The loss through the retirement of Dr. Richard Irwin as Associate Director of Laboratories was softened by the availability of Dr. Ernst Freese to assume that position. Dr. Freese, Chief, Laboratory of Molecular Biology has had a long and distinguished scientific career in NINCDS, has a good knowledge and firm understanding of its laboratories, and is highly respected for his integrity and administrative skills. The Institute is indeed fortunate that he is willing to be Associate Director for Laboratories as well as continuing as Chief of one of the Laboratories and has already benefitted from his wise counsel and useful suggestions in allocation of resources and appropriate nurturing of new resources.

The research programs encompass clinical investigations carried out primarily in the Branches and fundamental studies which are largely pursued in the Laboratories. The Scientific Director in his capacity as Associate Director for Branches and the Clinical Director are responsible for the supervision of the clinical research efforts, whereas the programs in the Laboratories are under the direct supervision of the Associate Director for Laboratories.

This summary of FY 85 will focus on the issues of personnel, space, and budget of the IRP, whereas the major scientific advances in the Laboratories and Branches are included in the summaries provided by the Laboratory and Branch Chiefs.

The major administrative concerns of the IRP during FY 85 have been in regard to space allocations to accommodate the newly appointed staff and to provide for new initiatives in research. The major changes in personnel which were outlined in the Scientific Director's summary of FY 84 necessitated a series of plans to most efficiently utilize the space resources of the Institute and integrate these with the renovations and moves which have been planned within the Clinical Center modernization program. These decisions about space have been only partially implemented; their extent requires several years and there have been slippages in the scheduling of the renovations and moves. It will not be informative to review in detail all of the relocations which have been planned, but it would be appropriate to outline some of the major considerations which have motivated these changes and to provide a broad picture of the final objectives.

The need for adequate animal research facilities to meet AALAC accreditation standards has taken the highest NIH space priority and this will impact on the neurosurgical operating rooms and on NINCDS facilities in Building 9 and in Building 36. Building 10A, which formerly housed two neurosurgical operating rooms, is to become a central facility for housing of animals for the whole of the Clinical Center. A new wing is to be added to Building 10 to accommodate additional operating rooms, including one for neurosurgery. We have been assured that space will be made available to accommodate offices and laboratory functions (electron microscopy, etc.) which are being displaced from Building 10A, but this space has not yet been identified.

In Building 36, a centralized animal facility is planned for the basement, but two areas (for Drs. London and Burke) have been allowed for special studies in primates. Building 9 has been redesigned to centralize primate housing and to ensure adequate space for surgical procedures and behavioral observations in primates. All of the plans for projected animal facilities have been developed with the cooperation and approval of Dr. Herbert Amyx, the Institute Veterinarian.

The laboratory space in Building 9 will be consolidated to provide a resource for a new Laboratory on Neuronal Regeneration and Implantation. Permission to establish this new laboratory has been requested. Many of its potential components are distributed in sections of the various laboratories and it is hoped that this laboratory will focus and coordinate these research efforts.

Dr. Mark Hallett, during this first complete year as Clinical Director has reorganized that Office to facilitate its various functions. The neurological consultative service led by Dr. Alison Wichman, Deputy to the Clinical Director, has attained a reputation for promptness and excellence. The EEG and EMG diagnostic services under Dr. Sato and Dr. Hallett, respectively, has also played an important role in providing consultative services to the other Institutes as well as NINCDS. They have been disadvantaged in that there have been delays in completion of the renovations of the fifth floor ACRF which was to accommodate these services. It is hoped that the appropriate moves will be possible in FY 86.

The educational program under the aegis of the Office of the Clinical Director has established a well attended weekly Grand Rounds held in the ACRF Amphitheater and the weekly Clinical Conference which deals primarily with issues of clinical care.

The Medical Neurology Branch with Dr. Roger Porter as Chief has five sections, including the Clinical Epilepsy Section (Dr. Roger Porter), Clinical Neuropharmacology Section (Dr. Ronald Polinsky), Neuropsychology Section (Dr. Paul Fedio), Neuronal Excitability Section (to be appointed), Human Motor Disorders Section (Dr. Mark Hallett), and a Speech Pathology Unit (Dr. Christy Ludlow). Dr. Ludlow has moved into space in the ACRF and is in the process of placing her computer assisted diagnostic equipment in an area contiguous to the NINCDS Outpatient Department on the fifth floor of the ACRF. Space for the Neuronal Excitability Section has been renovated and this laboratory is now being outfitted, although research is proceeding in temporary space. The Clinical Neuropharmacology Section is awaiting completion of renovations so that it can move from its temporary space to a permanent home on the North Corridor of the fifth floor of Building 10.

Planned renovations of the nursing units include installation of new audiovisual monitoring for epileptic and other neurological patients; space in the basement of Building 1 has been provided to accommodate the monitoring equipment and personnel which are part of the Clinical Epilepsy Section of the Medical Neurology Branch.

The Developmental and Metabolic Neurology Branch continues its research in laboratories in the Park Building as well as in the Clinical Center. Plans for renovation of the D-Corridor South on the fifth floor are being completed, but it appears unlikely that this renovation will be completed in FY 86. As anticipated, Dr. Edward Ginns was tenured and will continue his active research on cloning of genes for enzymes involved in lysosomal storage diseases. Dr. Norman Barton continues to be the focal person in daily care of the patients and is in a tenure-track position.

The Surgical Neurology Branch, headed by Dr. Paul Kornblith, has recruited Dr. Elizabeth Grimm who has set up a temporary laboratory in Building 9 and planned her new permanent laboratory on the fourth floor of the Clinical Center (Rooms 4N-246-252); renovations are planned for completion in FY 86. Dr. Richard Youle is also in temporary space and is in the process of planning his laboratories. These laboratory investigators recently joined the Surgical Neurology Branch and are involved in basic immunological research relevant to brain tumor control. Dr. Donald Wright has been tenured as a clinical investigator and will continue to pursue his studies on the blood-brain barrier. Dr. Edward Oldfield, who was tenured last year, is involved in studies of pituitary tumors and neural implants in brain tissue. There are several considerations regarding space which are awaiting resolution of the issues regarding Building 10A (see above) but a Neurological-Neurosurgical Special Care Unit has been completed in Nursing Unit 5W which can care for post-operative patients.

There have been no other plans for major shifts in the Experimental Therapeutics Branch nor the Infectious Diseases Branch.

The Biometry and Field Studies Branch has continued its two major efforts - the Data Banks and collaborative research efforts involving both the Extra- and Intramural Research Programs. The Neuroepidemiology Branch also continues its long-term projects, but has also initiated plans for participation in collaborative studies with other Laboratories and Branches in the Institute, e.g., defining high risk groups for study of Parkinson's Disease development.

Brain imaging is becoming increasingly important in neurological research and this is being reflected in the number of investigators who are expressing an interest in becoming involved more extensively in one or more of these techniques. Dr. Giovanni Di Chiro is Chief of the Neuroimaging Section in the Office of the Scientific Director and serves as a focal point for interactions of the Institute's Clinical Investigators and the two Departments (Nuclear Medicine and Radiology) involved in use of these techniques. It is clear that it will be useful for young NINCDS Investigators who are interested in exploiting PET or MRI to have an opportunity to participate actively in the Clinical Center Department activities and suitable arrangements are in progress to this end. The allocation of the PET resource is currently being coordinated by a Policy Advisory Committee (PET-PAC) of which the NINCDS Scientific Director is a member. It is anticipated that similar issues will require centralized regulation of Magnetic Resonance Imaging (MRI) resources and a central facility for MRI is being planned.

The Laboratory of Biophysics is located in facilities in Building 36, but also has a major division in Woods Hole at the Marine Biological Laboratory. Their space has been stable and no major changes are anticipated. Dr. John Clay has been awarded tenure to continue his research on membrane structure and ion channels.

The Laboratory of Experimental Neuropathology continues its focus on the mechanisms of demyelination in infectious neurological disorders and the possible relationships to multiple sclerosis. Dr. John Martin, who has been a major contributor to these studies has been awarded tenure.

Work on differentiation of mammalian cells has been initiated in the Laboratory of Molecular Biology and plans for relocating Dr. Henneberry from the Park Building to Building 36 are in progress and awaiting appropriate renovations.

As indicated earlier, space reallocations to accommodate projected needs to meet NIH standards for animal care have been planned in coordination with other Institutes in Building 36. These arrangements affect particularly the Laboratory of Neural Control. The Laboratory of Central Nervous System Studies has been assigned space in the basement of the Lister Hill Center to relocate its film library and some associated medical records as well as work area for visiting scientists. This move will free two laboratory modules for use in recruiting a new Chief for the Laboratory of Neuro chemistry. The rearrangements of laboratory space will serve to consolidate investigators who are currently located at a distance from facilities which they must use and will accommodate some recently recruited scientists in the Laboratory of Neurophysiology. The details of the various projected renovations and moves have been planned by Dr. Ernst Freese in cooperation with the various chiefs of the concerned laboratories.

The budget for the IRP has remained relatively stable and is deemed to be adequate to support the current research efforts, but high priority research on AIDS infection and the central nervous system may strain our resources. New initiatives in cellular biology and molecular genetics and the wider application of the techniques of these disciplines by investigators in the various branches and laboratories will place greater demands on the available fiscal and space resources. It may become necessary to limit spending in some areas and/or curtail research in programs which do not have the highest of priorities.

There were numerous awards given to IRP personnel during FY 85. Dr. Henry De F. Webster received the Alexander von Humbolt Award and will be visiting Germany to exchange information with scientists in that country. Dr. John Barranger received the Arthur S. Fleming Award for his research on Gaucher's Disease. Other outstanding performances of IRP staff were recognized in the following awards: Dr. Bruce Schoenberg received the Commissioned Corps Outstanding Service Medal, Dr. Jeffrey Barker received the Commissioned Corps Meritorious Service Medal, Dr. Gerald E. Loeb received the Commissioned Corps Commendation Medal, Dr. Clarence Gibbs received the NINCDS EEO Award and Dr. Paul Kornblith received the Superior Service Award.

TAB 1A -- OFFICE OF THE CLINICAL DIRECTOR -- (ODIR/OCD)

ANNUAL REPORT

May 4, 1984 through September 30, 1985

Office of the Clinical Director
Office of the Director, Intramural Research Program
National Institutes of Neurological
and Communicative Disorders and Stroke

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Annual Report: October 1, 1984 to September 30, 1985

Office of the Clinical Director

Mark Hallett, M.D., Clinical Director

With the arrival of Dr. Hallett as Clinical Director, on April 1, 1984, there has been a reorganization of the Office, which has been accomplished during this reporting period. In addition to handling administrative matters, mainly relating to patient care and coordination of educational activities, the Office has taken charge of delivery of neurological services. Service functions can be divided into the EEG Laboratory, the EMG Laboratory, the Consultation Service, Neuropathology and Paraprofessional Support Services.

The important administrative issues relate to planning for reorganization of the outpatient clinics to move to a permanent home on the fifth floor of the ACRF and initial programming for the renovations of the inpatient facilities on 5E and 5W. The two major educational conferences are the Clinical Conference (held on Tuesday afternoon) which is aimed at the Medical Staff Fellows and typically reviews a patient in detail, and the NINCDS Grand Rounds (held on Friday afternoon) which has been moved to the ACRF Amphitheater to accommodate the increased number of attendees. The Clinical Conference includes some attention to matters of patient care and quality assurance. The Grand Rounds continues to offer CME credit.

EEG Laboratory, Susumo Sato, M.D., Chief

Diagnostic Services:

The total number of tests performed during this reporting period increased by more than one hundred for the EEG examination and by more than one hundred and fifty for evoked potential tests (EP), compared to the last reporting period. This is a significant increase for a laboratory that has not changed the size of its workforce. The increase does not include some special electrophysiological monitorings that are described below, so that a rather sudden jump in the workload of the laboratory personnel can be appreciated. The major portion of the increase came from our Institute, the referrals from which exceeded more than 60% of the total examinations (previously about 50%).

	EEG		Evoked Potentials	
Referral Sources	No.	%	No.	%
NINCDS	399	35	226	47
OPD (NINCDS)	326	29	129	27
NIMH	115	10	2	1
NHLBI	23	2	11	2
NIADDK	35	3	6	1
NCI	68	6	13	3
NIAID	17	2	4	1
NIA	91	8	35	7
NICHD	58	5	4	1
Normal volunteer			48	10
Total (1610)	1132	100	478	100

Participation in research activity:

The EEG laboratory maintains a close tie with the Epilepsy Section of the Medical Neurology Branch and provides an invaluable diagnostic service in terms of localization of epileptiform discharges. The laboratory is capable of video recording events during the routine EEG recording and this capability often provides an invaluable observation in the research endeavors. The laboratory personnel prepare patients for PET scanning and magnetoencephalographic recording. The latter has been rather frequent. The laboratory personnel also assist in preparing the patients for, and in monitoring, subdural electrode recording, electrocorticographic recording during temporal lobectomy, intracarotid sodium amytal injection (Wade test), for locating the dominant hemisphere for speech, and sphenoidal electrode recording in patients with epilepsy.

The laboratory personnel also prepare test subjects for the psychology group for their evoked potentials.

The patients who were referred for the examinations were predominantly protocol-based. The timing of the examination was most critical in patients who underwent the protocol of gadolinium injection and magnetic resonance imaging (12 tests), and to some extent in familial Alzheimer patients (4-5 tests). The laboratory personnel have coped with these strict requirements well. Thirty-five EEGs were done in patients with Alzheimer's disease, who belonged to a different protocol.

The protocol of epileptic patients and evoked potentials involved 76 tests, including 48 in normal volunteers. Patients with multiple sclerosis, who underwent the treatment of cyclosporine, had 76 tests. Evoked potentials also were done in familial Alzheimer patients.

The EEG laboratory provides a training environment for Medical Staff Fellows toward the American EEG Board. The laboratory chief continues to serve as associate examiner for the Board.

Abstract: Sheridan, P., Sato, S., Forster, N., Bruno, G., Fedio, P., Chase, T.N.: Alpha background and parietal lobe function (Positron Emmission Tomography, Psychometry) in Alzheimer's disease. Annual Meeting American EEG Society, Salt Lake City, Utah, September 13-15, 1984.

EMG Laboratory, Mark Hallett, M.D., Acting Chief

EMG Activities (May 1984-June 1985)

Referrals	NINCDS	70
	Other Institutes	67
Collaborative study	Post-polio syndrome	10
	Dysautonomia	17
	Fabry's disease	5
	Polymyositis	2
Protocol 84-N-203	Hemifacial spasm	5
	Sarcoid	3
	Familial leukodystrophy	12
	In vivo action potentials	4
	Diagnostic dilemmas	35
	Normals	33
TOTAl		263

Professor F. Buchthal, who had been running the laboratory has retired, and Dr. Hallett is running it on an interim basis until a new permanent chief can be found. The laboratory takes referrals, is participating in a number of collaborative investigations, and conducts some independent research. Research activities are in an early stage.

In relation to collaborative ventures, for example, the neurophysiological investigation of patients with autonomic dysfunction has been undertaken. Seventeen patients with various forms of autonomic dysfunction and fourteen normals have undergone a battery of tests designed to evaluate the autonomic nervous system. Preliminary findings suggest that the neurophysiologic techniques are able to define and quantify autonomic dysfunction. Ten patients with progressive weakness, following acute polio, have been evaluated. All patients have undergone a detailed neurophysiologic assessment, the preliminary data indicating that polio is a chronic and persistent process of the motor neuron and that the abnormalities may arise in the motor neuron terminals. Five patients with Fabry's disease have been studied neurophysiologically and are normal, a finding contrary to reports in the literature.

The objective of one independent research protocol has been four-fold: first, to learn more about established diseases; second, to identify and characterize new diseases; third, to assess current methodologies and technologies; and fourth, to refine old methods and develop new ones.

Thus far, five projects have been initiated. Sarcoid polyneuropathy is a rare complication of systemic sarcoidosis. Scant information is available in the literature. Thus far, three cases of peripheral neuropathy have received detailed evaluations. The findings indicate that there is a distal, symmetric, sensory and motor polyneuropathy of axonal type. To date, this represents the largest series of sarcoid neuropathy with such detailed information.

Hemifacial spasm has recently been reported to have unique physiological features suggesting that ectopic excitation and ephaptic transmission are the primary mechanisms. Five cases of hemifacial spasm have been seen to date. Our findings corroborate recent reports and, further, have suggested that post-paralytic hemifacial spasm may have a similar pathogenesis. This raises the speculation that postparalytic synkynesis may be a result of ectopic excitation and ephaptic transmission rather than the presumed mechanism of aberrant regeneration.

Thirty-five cases of diagnostically difficult neuromuscular diseases have been referred to our laboratory for diagnostic evaluation. All patients underwent detailed evaluation. Nearly fifty percent of these cases have yielded satisfactory diagnoses of recognized but rare diseases. These diagnoses have included various metabolic conditions of muscle, unusual polyneuropathies, and disorders of neuromuscular junction transmission. Some of these cases are suitable for clinical reports. All have directly benefited the patient.

In assessing some of the current technologies, two studies have been undertaken. First, in evaluating the symmetry of the sensory action potentials, twelve cases have been studied. Preliminary results indicate that there is up to fifteen percent asymmetry of the sural action potential and nearly a fifty percent asymmetry of the peroneal sensory action potentials. Second, twenty-five cases have undergone comparison of the near-nerve and surface recording technique. Preliminary observations indicate that there is good correlation of the conduction velocity and nearly linear correlation of the amplitudes.

Four sural nerve biopsies have been evaluated by in vitro compound action potentials. In two conditions (autosomal dominant leukodystrophy and multisystem atrophy) the A-fiber populations, and in particular the C-fiber populations, were normal - which is consistent with the primarily CNS (preganglionic) involvement. In the other two conditions (demyelinating sensory neuropathy of unknown etiology and sarcoidosis) the A-fibers were involved without C-fiber involvement. The technique is a useful adjunct in the evaluation of nerve biopsies.

In a second research protocol, thoracic and abdominal evoked potentials were studied in five normal volunteers. The purpose of this study was to establish a method of somatosensory evoked potentials which would: (1) establish definition of normative waveform patterns and component overlying the regions of the scalp and spine upon electrical stimulation of both thoracic and abdominal nerves; (2) determine the central conduction times in normal controls upon electrical stimulation of both thoracic and abdominal nerves; (3) to define the optimal stimulation sites, intensities, and rates upon stimulation of the thoracic and abdominal nerve in normal volunteers. These goals have been accomplished. The findings should be useful for evaluation of patients with spinal cord dysfunction.

Publications:

Hallett, M., Tandon, D. and Berardelli, A.: Treatment of peripheral neuropathies. J. Neurol. Neurosurg. Psychiatry, 1985, in press.

Hallett, M.: Electrophysiologic approaches to the diagnosis of entrapment neuropathies. In: Aminoff, M.J. (Ed) Neurologic Clinics. Saunders, 1985, in press.

Wichman, A., Buchthal, F., Pezeshkpour, G., and Fauci, A.S.: Peripheral neuropathy in the hypereosinophilic syndrome. Neurology, 1985, in press.

Wichman, A., Buchthal, F. Pezeshkpour, G. and Gregg, R.: Peripheral neuropathy in Abetalipoproteinemia. Neurology, 1985, in press.

Krarup, C. and Buchthal, F. Conduction studies in peripheral nerve. Neurobehav. Toxicol. Teratol 7: 1-5, 1985.

Buchthal, F. Electrophysiologic aspects of myopathy. In: Aminoff, M.J. (Ed) Neurologic Clinics. Saunders, 1985, in press.

Consultation Service, Alison Wichman, M.D., Deputy to the Clinical Director for Consultations

The neurology consult service provides emergency and routine consultations for patients hospitalized in the Clincial Center and for outpatients. In the 1984 calendar year, 716 patients were seen, and from January through June 1985, 403 patients (inpatients and outpatients) have been evaluated. The outpatient population includes patients referred to the bi-weekly neurology consult clinic, and those seen in consultation in other clinics (i.e., oncology, immunology, cardiac surgery). Neurological follow-up of patients is arranged as needed.

Publications:

McKeever, P., Wichman, A., Chronwall, B. and Howard, R.: Sarcoma grows from human glioblastoma. South. Med. J. 77(8): 1027-1032, 1984.

Brin, M.F., Gregg, R.E., Pedley, T.A., Lovelace, R.E., Wichman, A., Behrens, MM., Gouras, P., MacKay, C., Kayden, H.J., Baker, H., and Levy, J.: Vitamin E deficiency and neurologic disease: clinical and electrophysiologic evaluation in 24 patients. Abstract. XIIIth World Congress of Neurology, Hamburg, Germany, September 1-6, 1985.

Dr. Marinos Dalakas has performed muscle and nerve biopsies in the operating room under local anesthesia for the investigation of neuromuscular symptoms of patients from different Institutes. The biopsied specimens are processed in the histochemistry laboratory we have established, for a battery of 14 histochemical reactions. He has also processed or reviewed several muscle biopsy specimens sent to us from outside hospitals for expert advice. He has also seen in consultation several patients with neuromuscular complaints, admitted and studied by investigators of other Institutes. Specifically, he has been studying patients under approved clinical protocols in collaboration with: 1) Paul Plotz, M.D., NIADDK, for the study of patients with polymyositis; (2) William Gall, M.D., NICHD, for the study of patients with cystinosis and carnitine deficiency; 3) Michael Frank, M.D., NIAID, for the study of patients with hereditary angioedema and muscle pains; and (4) Neal Cutler, M.D., NIA, for the study of neuromuscular changes in normal aging and patients with Alzheimer's disease. In addition, we examine, in the outpatient clinic and study as inpatients, several patients admitted under NINCDS clinical protocols for the study of patients with postpoliomyelitis, muscular atrophy, peripheral neuropathies and amyotrophic lateral sclerosis. Progress in his research activities is reported with the Infectious Diseases Branch.

Neuropathology, David A. Katz, M.D.

Diagnostic neuropathology services for NINCDS, and for all other Institutes, are provided by Dr. Katz. The neuropathology service is integrated with both the Autopsy and Surgical Pathology Sections and residency training program of the Laboratory of Pathology, NCI. The brain is examined in approximately 100 of the 150 autopsies performed at NIH each year; fully 25% of these manifest significant primary or secondary neurological disease. Braincutting is held weekly and relevant neuropathological findings are also presented at gross autopsy conferences. Selected cases are further utilized for neurological clinical conferences. Neurosurgical specimens include both in-house and submitted material, for an annual total of approximately 175 cases; intra-operative frozen-section consultations are required in approximately 35 in-house cases per year.

The neuropathology service also functions in a collaborative manner to provide subspecialty expertise in a range of clinicopathologic investigations. A study of urokinase treatment of intraventricular hemorrhage in rabbits, conducted by Dr. Raj Narayan (SNB, NINCDS) was recently published (Narayan R. et al., J. Neurosurg. 62: 580-586, 1985). Currently in progress are (1) reviews of the pathology of glioma patients treated under NIH protocols with spirohydantoin mustard (SNB, NINCDS), and with IuDR radiosensitization (ROB, NCI), and (2) clinicopathologic case studies including several unusual neurological manifestations of AIDS, glioblastoma with systemic metastases, and a Menkes-like disorder of copper metabolism.

Paraprofessional Support Services

Linda Nee, MSW, is assigned to the Clinical Neuropharmacology Section, Medical Neurology Branch. She has been pursuing clinical and family studies, organizing field clinics and undertaking genetic counseling.

Helen Krebs, RN, is assigned to the Neuroimmunology Branch where she is taking a major role in running a clinical trial of the use of cyclosporine in multiple sclerosis.

Marjorie Gillespie, RN, is assigned to the Experimental Therapeutics Branch where she supports several aspects of the clinical program.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02675-01 ODIR

PERIOD COVERED

May 4, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Evaluation of Neuromuscular Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Mark Hallett, M.D.	Clinical Director	OCD ODIR	IRP	NINCDS
Others:	John Ravits, M.D.	Medical Staff Fellow	OCD ODIR	IRP	NINCDS
	Michael Baker, M.D.	Medical Staff Fellow	OCD ODIR	IRP	NINCDS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Office of the Clinical Director, Office of the Director, Intramural Research Program

SECTION

EMG Laboratory

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The revolution in the understanding of neuromuscular diseases has been founded on careful clinical observation, neurophysiologic technologies, and histopathology. This protocol has been carried out to learn more about established diseases, to characterize new diseases, to assess current methodologies and technologies, and to refine old methods and develop new ones.

To date, three patients with sarcoid neuropathy have been evaluated and have been shown to have a distal symmetrical sensory and motor polyneuropathy of axonal type. This is the largest series to date. Five patients with hemifacial spasm have been evaluated and findings indicate that the primary abnormalities may be generated by ectopic excitation and ephaptic transmission of axons in the peripheral facial nerve. These findings corroborate those which have recently been detailed in the literature. In addition, the findings suggest that a similar pathogenesis may be involved in post- paralytic facial abnormalities. Thirty-five patients with diagnostically difficult neuromuscular diseases have been evaluated and nearly fifty percent of them have yielded satisfactory diagnosis. Several of these cases are suitable for clinical reports. All of the findings have directly benefited the patients. Fourteen patients have undergone a critical assessment of the symmetry of the sensory action potentials and preliminary findings indicate that significant asymmetries of sensory action potentials may exist. Twenty-five cases have had direct comparison of near-nerve recording and surface recording techniques and preliminary findings indicate good correlations of the techniques. Four cases of nerve biopsy have been studied with in vitro compound action potentials and this is a useful adjunct technique in the evaluation of nerve biopsies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02676-01 ODIR

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Thoracic and Abdominal Somatosensory Evoked Potentials in Normal Volunteers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Mark Hallett, M.D.	Clinical Director	OCD	ODIR	IRP	NINCDS
Other:	Michael Baker, M.D.	Medical Staff Fellow	OCD	ODIR	IRP	NINCDS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Office of the Clinical Director, Office of the Director, Intramural Research Program

SECTION

EMG Laboratory

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Thoracic and abdominal evoked potentials were studied in five normal volunteers. The purpose of this study was to establish a method of somatosensory evoked potentials which would: (1) establish definition of normative waveform patterns and component overlying the regions of the scalp and spine upon electrical stimulation of both thoracic and abdominal nerves; (2) determine the central conduction times in normal controls upon electrical stimulation of both thoracic and abdominal nerves; (3) to define the optimal stimulation sites, intensities, and rates upon stimulation of the thoracic and abdominal nerve in normal volunteers. These goals have been accomplished. The findings should be useful for evaluation of patients with spinal cord dysfunction.

TAB 1B – NEUROIMAGING SECTION – (ODIR/NIS)

ANNUAL REPORT
October 1, 1984 through September 30, 1985

Neuroimaging Section, OD, IRP
National Institute of Neurological and Communicative Disorders and Stroke

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Z01 NS 02315-08 NIS

ANNUAL REPORT
October 1, 1984 through September 30, 1985
Neuroimaging Section, ODIR, IRP
National Institute of Neurological and Communicative Disorders and Stroke

Giovanni Di Chiro, Chief

I. SUMMARY

Following is a summary of the major findings for the research protocols of the Neuroimaging Section in the fiscal year October 1, 1984 through September 30, 1985.

Radiographic and Radioisotopic Angiography of the Spinal Cord. (Project #Z01 NS 01195-21) Angiographic studies of arteriovenous malformations and vascular tumors of the spinal cord have continued. Digital subtraction angiography (DSA), either intravenous or intraarterial, has not proven to be particularly reliable. More useful, at least for the recognition of the vascular nature of these lesions, has been the technique of dynamic computed tomography (DCT).

Computed Tomography (Transmission) and Nuclear Magnetic Resonance (NMR). (Project #Z01 NS 02073-12) CT studies of such conditions as degenerative diseases of the CNS, cavities of the brain stem and spinal cord, and brain and spinal cord tumors have continued.

The NMR imaging research has developed along several lines:

1) Taking advantage of the exquisite NMR display of morphological detail to advance the diagnostic yield in a number of neurological lesions.

2) Carrying out a study of a large group of patients with tumors and arteriovenous malformations of the spinal cord: This study represents the first assessment of the NMR imaging capabilities in one area of spinal cord pathology.

3) Trying to learn more about the NMR signal of various abnormal tissues. Particular attention has been devoted to the signal from CNS tumors of various types and grades and from intracranial, extravasated blood. We are also engaged in a comparative "in vivo - in vitro" study of T_1 and T_2 of normal and pathological CNS tissues.

4) Comparing clinical NMR imaging results with those of CT and particularly PET in a variety of abnormal conditions, starting with CNS tumors.

5) Developing an experimental method (monkeys) for MRI cisternography and myelography using Gd-DTPA.

Positron Emission Tomography. (Project #Z01 02315-08) The experience with PET-FDG of CNS tumors has continued to expand. About 300 patients have been studied and in many cases repeat examinations have been performed. The usefulness of the PET-FDG for grading cerebral tumors is well established. This technique has also been used to predict the survival rate of patients with high grade cerebral gliomas. PET-FDG is by far the best method to establish the prognosis in these patients.

An analysis of the cortical glucose metabolism in the hemianopsias starting with the homonymous field defects has been initiated. In cases of hemianopsia, the appropriate primary and associative visual cortices show marked hypometabolism.

A long range research project to compare PET with NMR - in tumors, epilepsy, and degenerative diseases - has begun. Preliminary observations indicate that the two techniques complement each other.

Finally, PET studies of dopamine and opiate receptors have been carried out in a group of monkeys. Dopamine receptors have been analyzed with $[^{11}C]$3-N-methylspiperone and opiate receptors with $[^{18}F]$3-acetylcyclofoxy. It appears that the anatomo-functional distribution of these ligands in the thalami and basal ganglia is different and distinctive.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 01195-21 ODIR

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Radiographic and Radioisotopic Angiography of the Spinal Cord

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
Giovanni Di Chiro, M.D. Chief, Neuroimaging Section, ODIR, NINCDS

OTHER:

J. L. Doppman	Chief	DRD, CC
E. H. Oldfield	Senior Staff Physician	SN, NINCDS
S. M. Larson	Chief	NM, CC

COOPERATING UNITS (if any)
Diagnostic Radiology, and Nuclear Medicine Departments, Clinical Center, NIH; Medical Examiner's Office, Department of Public Health, Philadelphia, PA

LAB/BRANCH
Office of Director, IRP

SECTION
Neuroimaging Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Selective arteriography (radiographic) of the spinal cord is a diagnostic technique which has proven to be informative in cases of arteriovenous malformation (AVM), tumor, obstructive vascular disease, trauma, and postradiation damage of the spinal cord.

Radioisotope angiography of the spinal cord offers some advantages as a screening method, and in certain types of intraspinal pathology may give information not available by any other diagnostic test.

Experience with the techniques of dynamic computed tomography, (DCT), digital subtraction angiography (DSA), positron emission tomography (PET) using ^{18}F-2 deoxyglucose and nuclear magnetic resonance imaging (MRI) of the spine indicates that these methods may be useful screening and follow-up procedures in the evaluation of certain vascular lesions and tumors of the spinal cord.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02073-12 ODIR

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Computed Tomography (Transmission) and Nuclear Magnetic Resonance (NMR)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
Giovanni Di Chiro, M.D. Chief, Neuroimaging Section, ODIR, NINCDS

OTHER:	R. A. Brooks	Staff Physicist	NIS, NINCDS
	D. S. Fishbein	Staff Fellow	NIS, NINCDS
	D. Bairamian	Visiting Associate	NIS, NINCDS
	M. M. Polsby	Staff Fellow	NIS, NINCDS
	E. J. Finn	Research Physicist	NIS, NINCDS
	J. L. Doppman	Chief	DRD, CC
	S. M. Larson	Chief	NM, CC

COOPERATING UNITS (if any)
Diagnostic Radiology, and Nuclear Medicine Departments, Clinical Center, NIH.

LAB/BRANCH
Office of the Director, IRP

SECTION
Neuroimaging Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.7	

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Computed Tomography in its transmission (CT), emission (PET, SPECT), and Nuclear Magnetic Resonance (NMR) modalities, represents the main research area of the Neuroimaging Section.

CT: Ongoing clinical and experimental research projects in transmission CT, include studies of tumoral, degenerative, demyelinating and atrophic processes of the brain, plus hydrocephalus, brain edema, postradiation cerebral necrosis, and surgically correctable lesions in young patients affected by chronic epilepsy.

NMR: Our NMR imaging research is developing along five main lines: 1) we are taking advantage of the exquisite capability of NMR to display fine anatomical detail to advance our diagnostic yield in a number of neurological lesions; 2) we are trying to learn more about the NMR signal of various tissues (in vivo and in vitro studies), starting with the signal from extravasated blood (various types of CNS hemorrhages) and the signal from various types-grades of CNS tumors; 3) we are comparing our clinical NMR imaging results (emphasis on spinal cord diseases, brain tumors, degenerative diseases, and complex partial epilepsy), with those of CT and particularly PET in a variety of abnormal conditions; 4) studies on contrast media (Gd-DTPA) for MRI to be introduced either systemically or intrathecally; and 5) developing new NMR imaging strategies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02315-08 ODIR

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Positron Emission Tomography

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
Giovanni Di Chiro, M.D. Chief, Neuroimaging Section, ODIR, NINCDS

OTHER:

R. A. Brooks	Staff Physicist	NIS, NINCDS
D. S. Fishbein	Staff Fellow	NIS, NINCDS
M. M. Polsby	Staff Fellow	NIS, NINCDS
E. J. Finn	Staff Physicist	NIS, NINCDS
D. Bairamian	Visiting Associate	NIS, NINCDS
N. J. Patronas	Staff Physician	DRD, CC

COOPERATING UNITS (if any) NM, CC, NIH; BIEB, NIH; Naval Res. Lab., Wash., D.C., Nat'l. Bur. of Standards, Wash., D.C.; LCM., NIMH, NIH; DRD, CC, NIH; ODIR, NINCDS, NIH; ETB, NINCDS, NIH; Brookhaven National Lab., Upton, NY; Div. of Nucl. Med., Dept. of Radiol., Johns Hopkins Univ. School of Med., Baltimore, MD.

LAB/BRANCH
Office of Director, IRP

SECTION
Neuroimaging Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Positron Emission Tomography (PET) using (^{18}F)-2-deoxyglucose (FDG) allows us to obtain anatomical data (e.g., axial transverse or coronal images of the brain) as well as dynamic functional data (such as regional cerebral glucose consumption rate, and measurement of storage, degradation and turnover of tagged metabolites). Besides FDG, other radiopharmaceuticals (e.g., those tagged with ^{15}O, ^{11}C, ^{13}N) can be used with PET to study the BBB, oxygen metabolism, protein synthesis, and, lately, the neuroreceptors. The unique property of PET is that it provides physiologic and pathophysiologic information not available with any other imaging procedure.

Since June 1982 we have been using the new high-resolution, high-sensitivity scanner built in our section, the NeuroPET. The performance of this scanner has exceeded all our expectations. This device has allowed new applications of the PET technique.

OTHER - *Continued

S. M. Larson	Chief	NM, CC
W. H. Theodore	Neurologist	EB, NINCDS
T. N. Chase	Chief	ETB, NINCDS
E. H. Oldfield	Staff Physician	SN, NINCDS
D. Wright	Staff Physician	SN, NINCDS
C. V. Kufta	Staff Physician	SN, NINCDS
M. Hallett	Clinical Director	IRP, NINCDS
R. J. Polinsky	Staff Physician	MN, NINCDS
A. P. Wolf	Senior Chemist	Brookhaven
L. Sokoloff	Chief	LCM, NIMH

TAB 1C -- INSTRUMENTATION AND COMPUTER SECTION -- (ODIR/ICS)

ANNUAL REPORT

October 1, 1984 - September 30, 1985

Instrumentation and Computers Section

National Institute of Neurological and Communicative Disorders and Stroke

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INSTRUMENTATION

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INSTRUMENTATION & COMPUTERS SECTION

National Institute of Neurological and Communicative Disorders & Stroke

October 1, 1984 - September 30, 1985

The Instrumentation and Computers Section provides technical support for investigators of NIMH and NINCDS IRPs by (1) assessing the instrumentation and computer needs of the investigator; (2) designing, developing and constructing special-purpose electronic and mechanical instrumentation and systems not commercially available; (3) designing, specifying and managing laboratory computer systems for data acquisition and processing.

Additional services provided by the Section include consultation on measurement techniques, signal processing, noise and electro-magnetic interference in data measurement systems, and equipment purchases. Several formal and informal courses for investigators are taught by Section personnel; topics include electrical circuit theory, operational amplifier applications, digital logic design, and computer applications.

Due to manpower limitations and economic considerations, the Section is unable to provide the following services: repair of commercial instruments, duplication of off-the-shelf commercially available equipment, and fabrication of non-instrument items (shelves, bookcases, etc.).

When an investigator requires the services of the Section, he first meets with the Section Chief and other personnel as needed to discuss his requirements. On the basis of this meeting, a decision is made as to whether ICS (Instrumentation and Computers Section) will take on the project. If a commercially produced instrument will satisfy the investigator's requirements, he is advised to purchase it. If custom instrumentation is needed, ICS will accept the project unless we lack the appropriate expertise, or our current work backlog is excessive. In these cases the project may be contracted to a private firm, or the investigator may be directed to the Biomedical Engineering and Instrumentation Branch (BEIB).

When the Section Chief or the Assistant to the Chief agrees to accept a project, the investigator submits a standard work request form (available from ICS), signed by his Lab Chief. This form will state the nature of the instrument or service requested, and will contain as many details and specifications as the investigator can provide.

The project is then assigned to an engineer, who will confer with the investigator to formulate a set of engineering specifications and a timetable and cost estimate for the project. The ICS does not charge for services, but the investigator will be billed for the cost of the components used. Upon delivery of the completed instrument, a memo is sent to the investigator listing the component costs and asking permission to have the Administrative Officer transfer funds from his CAN to the Section's CAN.

INSTRUMENTATION

The Section has a staff of five engineers and five technicians to design, develop, and fabricate electronic and mechanical instruments. The major effort is in the production of electronic instruments for basic neurophysiological research, and for clinical studies involving affective disorders. The following are brief descriptions of representative projects, chosen from a total of 238 projects undertaken this year.

(1) Patient Activity Monitoring System. The Section has continued to develop the Patient Activity Monitor (PAM) and the support hardware and software which forms the system.

(a) Monitor. The current version of the PAM has a memory capacity of 1024 locations and is in its third year of production. Fabrication, testing, and calibration of a set of 60 units begun last year has been completed and another set of 30 monitors is now in the final phase of fabrication. Most of the older versions of the PAM have now been retired. Approximately 100 monitors are in use, with the Section providing battery changes and repairs as needed. The injection-molded plastic case developed for the monitor last year is now in wide use. Early field tests revealed problems with static electricity interference. Coating the exterior of the case and end cap with a metallic conductive paint eliminated this problem. Compared to the older metal case, the plastic case reduces the overall weight of the monitor by 30%, provides a more water-resistent cover, and is less expensive and easier to produce.

(b) Telecommunications. A remote readout terminal for the patient activity monitor has been developed and is now ready for field evaluation. The terminal has the capability to be used in the home of a subject or in an office/laboratory environment. Using its internal modem, the terminal first dials a remote computer facility, then reads the contents of an activity monitor, sends the activity data over the phone lines, clears the monitor's memory, and hangs up. Initially, the VAX computer managed by the Section will be the remote (recipient) computer. Software has been written to reformat the data from the VAX into standard PDP-11 activity files for further analysis.

(c) Computer Support. A PDP-11/23 minicomputer with a 20 Mbyte hard disk is being prepared as a second PAM readout station for PAM users in Bldg. 10. This powerful system will handle all of the PAM software, eliminating the present dependency on the Bldg. 36 11/34 system for some of the more complex analysis programs. Software is also being developed to reformat activity files to allow direct transfer into WYLBUR files for statistical analysis. A new PAM computer interface using the RS-232-C serial data format is under development. This interface will allow an inexpensive personal computer with a serial port to serve as a readout device. The Section will develop software for the Apple Macintosh personal computer to support this readout method. This combination will allow individual laboratory readout stations and will also facilitate collaboration with groups outside the IRP.

(2) Neurophysiological Data Preprocessor. A microprocessor system has been developed to replace the custom logic circuitry presently used by the Laboratory of Neurophysiology Data Acquisition System. This preprocessor records the times of occurrences of 64 different events and eight different pulses. This information is transmitted to the main processor (a PDP-11 minicomputer) through a parallel interface and the information is coded in such a form as to ensure compatibility with existing software that is used for analysis and display of the data. The preprocessor decreases response time to events and pulses and it frees the main processor for experiment control. Following complete lab testing of the first unit fabricated last year, the Section constructed five additional units for the LNP/NIMH and two units for the LNLC/NINCDS.

(3) Rodent Activity System. A system was completed and is currently being used to monitor the running wheel activity of 72 rodents. Running wheel activity is important in circadian rhythm studies involving light response to free-running hamsters. Six surplus tissue culture boxes were modified to hold 12 cages each (2 on each of 6 shelves). The wheel activity in each cage is recorded with a simple microswitch and interface logic controlled by a 16-bit Plessey 6100 laboratory computer. In addition to the 72 running wheels, 36 fluorescent lights present a programmed light stimulus to each shelf (two cages). The computer monitors each light by means of a photodetector to provide verification that the lights were on at the proper time. The activity

and light data are stored on a 10 megabyte Winchester disk and also on two 1 megabyte floppy disks. In addition, data from all 108 channels is continuously plotted in 15-minute intervals on a printer/plotter in a strip chart format. The data for each cage is stored in continuous files to permit analysis using existing activity profile programs.

(4) Microprocessor-based Rotometer. A third generation animal rotation monitor was completed that utilizes an inexpensive microcomputer board to determine the clockwise or counter-clockwise rotations of one to four rodents in cylindrical cages and to hold this data for input into the serial port of a Macintosh computer. The computer board uses the 16-bit Intel 8088 microprocessor and has an on-board BASIC interpreter for fast program development. A second logic control board designed by ICS collects the rotational data using FIFO buffers until it is processed by the 8088. Software is being written which will use the capabilities of the Macintosh to store the data on disk, and to display the data in real-time histogram form.

(5) Biotelemetry Temperature Measurement. A microprocessor-based instrument is being developed to continuously monitor the body temperature telemetered from laboratory rodents. Data from an implanted commercial miniature transmitter is converted by a standard AM receiver into a series of pulses whose period is inversely related to temperature. The instrument will derive the actual temperature values from a memory calibration table and then display the result with 0.1 degree centigrade resolution.

(6) EEG Amplifier System. A second 32-channel EEG amplifier system has been completed for use in several ongoing research projects including topographic brain mapping. The design incorporates state-of-the-art integrated circuit components and printed circuit board layouts to produce a reliable, compact, low-cost-per-channel unit. Each channel consists of a preamplifier, amplifier, and a selectable antialiasing filter. A flexible design and front panel switches allow control over signal bandwidth, monitoring by a tape recorder and a 16-channel Grass polygraph, and digitizing and analysis of the EEG signal by a computer. A related project involved the design and construction of three EEG calibrators. By generating an 8 hertz, 100μvolt signal simultaneously on each of the 32 channels, this device allows system calibration and verification that all channels are working prior to a recording session.

(7) 32-Channel Analog Interface. Speech pathology studies will utilize a multiplexed A/D converter controlled by a PDP-11/73 minicomputer to digitize speech, muscle, and neuronal analog signals. To maximize the digitized signal-to-noise ratio, a compact 32-channel analog conditioning instrument has been developed. Each channel provides adjustable gain/attenuation, a selectable-bandwidth antialiasing filter, and a sample/hold amplifier. Each signal level is also displayed on a 10-segment LED VU meter so that its amplitude can be optimized before the A/D conversion. Printed circuit board construction and a modular packaging system were used to simplify fabrication and to increase reliability.

(8) Data Acquisition Computer Interface. A third generation interface device for data acquisition and control has evolved in several IRP laboratories. This instrument provides the interface between the experiment and the A/D and D/A boards within a PDP-11 minicomputer. The Section has designed and fabricated six of these interfaces this year. A companion 4-channel signal-conditioning system was delivered with three of these units. The companion instrument provides four decades of adjustable gain, selectable high and low frequency filtering, and adjustable input offset capability.

(9) Pulse Generator System. A multi-channel timing instrument (pulse generator system) is a vital part of many neurophysiological experiments. Instruments used within the IRP that were purchased about 15 years ago are no longer manufactured and have become somewhat unreliable.

Newer, commercially available units lack the flexibility and convenience of the older devices. Last year ICS designed a five-channel pulse generator system to fill this void. By employing both analog and CMOS digital design techniques, an instrument with both the required technical specifications and a high degree of operator convenience was realized. Six of these instruments were fabricated last year and an additional five units are currently under construction.

(10) Ambulatory Lux Monitor. An ambulatory data acquisition system (Vitalog PMS-8) is being used to monitor the temperature of manic-depressive patients. To allow simultaneous recording of the ambient light levels experienced by these patients, a small, micro-power lux meter is being developed as an input transducer for the PMS-8. Several photodiode/ logarithmic amplifier combinations are being evaluated to obtain a five decade photometric response. The microprocessor data processing algorithms employed in the PMS-8 and in the readout Apple II computer are being modified to collect, convert, and store the light intensity data.

(11) Microdensitometer. A standard split-viewing Zeiss compound microscope has been converted into a microdensitometer that produces a density reading from a small central spot (selectable as either .25, .63 or 1.6mm dia.) within the 18mm diameter viewing area. A linear photodiode/amplifier combination converts the light transmission value within the spot into a proportional voltage for a microprocessor-controlled A/D converter. Corresponding to each transmission value, a logarithmic density value is obtained from a memory look-up table. Transmission and density values are simultaneously displayed and the density value may be printed to facilitate recording of numerous successive readings. The split-viewing ability of the microscope allows precise areas on the autoradiographic film to be identified by simultaneously viewing the film and a stained slide of the same brain slice section.

(12) 30-Channel Electrode Array Amplifier System. A complete system for amplifying and processing signals from a micro-miniature array of 30 gold electrodes is being used in a variety of experiments to record cultured nerve tissue cell interactions. The neural signals are preamplified on a circuit board that also serves as a base for the electrode array holder. The preamplifiers connect to the main amplifier/discriminator units which provide three settings for overall gains of 100, 1,000, or 10,000. Each amplified signal is fed to a comparator with a front panel adjustable threshold level. The comparator output triggers a one-shot which is latched and sampled by a computer. Additionally, a multiplexer is provided to display the amplified signal, comparator level, and one-shot output on a single output of an oscilloscope. Use of printed circuit cards for both the preamplifier and amplifier units and a modular packaging system greatly simplified the fabrication and increased the system reliability.

COMPUTERS

Small computers are ideally suited for laboratory research in neurophysiology and psychology. They are used in the laboratory for on-line, real-time interactions, process control, and data acquisition. Recorded data may be stored, combined with other data, reduced statistically, transferred to larger computers for further analysis, transformed for presentation graphically or mathematically, and the results may be printed or plotted. Increasing use is being made of the small computer for processing the text of scientific papers and communications. Data base management is now available for the small computer, as are limited management information systems.

Techniques have been developed for image processing which are applicable to many diverse experimental systems, ranging from autoradiographs of brain tissue sections to the analysis of two-dimensional electrophoresis gels.

Larger minicomputers, the so-called super-mini's, have been reduced in price and are now available for functions formerly performed by larger time-shared systems. These systems allow applications in modeling, curve fitting and statistical treatment that would be prohibitively expensive on large systems.

Inexpensive personal computers are proving useful for dedicated applications. Many scientists are developing software for these computers, which they offer to the scientific community at low cost. PCs will become increasingly useful in the laboratory and their potential should be exploited.

Microcomputers incorporated in the design of biomedical instrumentation provide a savings in design and fabrication time for instruments, and a more flexible system than one based on discrete components.

The Instrumentation and Computers Section is actively involved in the applications of small computers in the IRP. By integrating the functions of biomedical instrument design and laboratory computer systems with software designed specifically for the research community, the Section offers computer support services for a broad range of scientific disciplines.

LABORATORY COMPUTERS

The design goal for the laboratory instrument computer is to provide maximum function, tailored to the specific experimental design, with minimum cost. ICS provides consultation on the specification and selection of laboratory computers for new applications; conducts systems studies in collaboration with the scientist; and helps the scientist in the procurement, installation and maintenance of the equipment.

In support of these efforts, ICS has maintained two PDP-11 central computers, one in Bldg. 36, and one in the Clinical Center. The functions previously provided by these computers are now being largely obtained by newer computer systems. The multi-user VAX-11/750 managed by ICS in Bldg. 36 provides high-capacity data storage, and efficient data processing, including graphic functions with plotting and printing on a high-resolution laser printer. Additionally, large price reductions have permitted individual IRP laboratories to acquire higher performance minicomputer systems which are self-supporting. Due to these developments, the central PDP-11/40 in the Clinical Center has been retired and the PDP-11/34 in Bldg. 36 is being replaced with a more powerful LSI-11/73 with significantly reduced maintenance costs. In addition to use for program development and training, the 11/73 will be equipped as a video image processing system.

TRAINING AND SOFTWARE SUPPORT

ICS provides training for the scientist or support personnel who will be programming and maintaining the system. Personnel limitations make it difficult for ICS to provide complete programming for specific individual applications, so such programming must be supplied by the laboratory. ICS computer personnel are always available for consultation, training, and help in debugging, as well as assistance in the selection of part-time programmers or consultants. Commercial software packages or applications from other research labs are often available, and ICS will evaluate such systems.

ICS develops and maintains a library of procedures which are written specifically for the laboratory computers used in the intramural community. These procedures are designed to be incorporated into the users' programs. In addition, ICS will aid the investigator in writing the difficult time and data dependent sections of real-time programs. ICS also develops some application programs which will have wide use within one or more laboratories or will support data acquisition hardware developed by ICS.

PROGRAM MAINTENANCE

There are now more than 60 minicomputers in the program; many of these systems have been in use for years. A significant number of library procedures and general-purpose application programs are used on these machines. As experimental protocols develop and change, software changes are often required, so program maintenance is a continual and time-consuming function of the Section. This effort is aided by structured programming techniques and standardization of laboratory computers and peripheral equipment.

VAX COMPUTER SYSTEM

The Section manages a multi-user VAX-11/750 computer system that is available for use by all investigators in the IRP. The VAX is located in Bldg. 36, in space furnished by the Laboratory of Cerebral Metabolism, NIMH. Potential users in Bldg. 36 may request installation of hard wired cable connections, or the VAX may also be used on a dial-up basis.

A device independent graphics package (PLOTLIB) has been developed on the VAX that permits plots to be generated on numerous display terminals and hardcopy devices. A terminal emulation program (TEM) was developed which permits small PDP-11 laboratory computers to function as graphics terminals when using the VAX. TEM also supports file transfers in both directions. A similar terminal emulation program is available for the Macintosh personal computer.

A TALARIS laser printer has been installed on the VAX which now permits publication quality plots and documents to be quickly and easily generated. The PLOTLIB graphics package was updated to support the laser printer and a program (FPRINT) has been written to allow documents incorporating superscripts, subscripts, and Greek letters to be printed on the laser printer.

IMAGE PROCESSING

The Section on Instrumentation and Computers maintains a general purpose image processing system consisting of an Optronics rotating drum film scanner, a Gould/DeAnza image array processor, and a PDP-11/60 computer. Images to be processed may be obtained by scanning autoradiographs, x-ray film, or photographic negatives, or by using images generated by CAT or ECAT scanners. A camera station is available to generate color hardcopy using Polaroid SX-70 or 35mm film.

Software packages that are easy to learn and use have been developed to provide an extensive and expandable repertoire of basic image processing functions. Special purpose functions can be developed to meet specific user requirements. The facility is useful for numerous applications involving evaluation and quantification of biomedical images . The two primary applications of the system are the densitometric analysis of autoradiographs of brain or tissue sections and the analysis of two-dimensional electrophoresis gels.

The Section is developing a new PDP-11/73 based image processing system that will be capable of using these software packages. This system will use a TV camera for digitizing images instead of the rotating drum film scanner. Unlike the drum scanner which can only digitize transparencies, the TV digitizer will permit any object that can be placed under a camera to be digitized.

PERSONAL COMPUTERS

The Section has evaluated Apple Macintosh personal computers for potential use in both scientific and administrative applications. The Macintosh was chosen for its ease of learning, advanced design, and high quality graphics. It has proven to be useful in a number of areas and is remarkably easy to use.

The most popular use of the Macintosh has been for scientific word processing. It has proven to be a very cost-effective alternative to expensive and inflexible dedicated word processors. It can easily produce text containing equations, Greek letters, superscripts, and subscripts. In addition, it can also produce posters or camera-ready charts for slides. When used with the new Apple Laserwriter printer, print quality is as good, or better, than that produced by a dedicated word processor.

The Macintosh has also proven useful as a graphics workstation for use with the VAX. An inexpensive program (VERSATERM) allows the Macintosh to function as either a VT100 compatible full screen editing terminal or as a Tektronix 4014 compatible graphics display terminal. Both text and graphics generated by the VAX can be printed on the Macintosh printer. In addition, text files can be transferred in both directions. The Macintosh also functions well as a terminal with other host computers such as WYLBUR, DECSystem-10, and MEDLINE.

The Macintosh is being used in three Section projects for low-speed laboratory data acquisition and control. The first project involves presenting stimuli (various words or geometric designs) to Alzheimer's patients with recording of patient responses. A second project uses the Macintosh to control and collect data from an HP 8450 Spectrophotometer. The third project uses the Macintosh to log data generated by a four-channel rodent rotometer developed by ICS.

MICROPROCESSORS

ICS also maintains a microprocessor development system for the software and hardware development of microprocessor-based instrumentation at both the chip and single board computer level. The system currently supports three common microprocessors; one 16-bit processor, and two 8-bit processors. These microprocessors and their associated peripheral chips are now available in CMOS low power versions. This development allows the design of both smaller, more reliable bench instruments and more intelligent portable instrumentation. The Section is evaluating a computer board which uses the 16-bit processor (Intel 8088) and comes with an on-board BASIC interpreter. This combination allows rapid software development and has already proved useful in low-speed data acquisition applications.

ENGINEERING, COMPUTER AND FABRICATION SERVICES

This table shows the distribution of the Section's workload among the various laboratories and branches. We have listed only the major users.

LABORATORY OR BRANCH	HOURS	PERCENT
Clinical Psychobiology, NIMH	2981	14.07
Neurophysiology, NINCDS	1493	7.05
Neurophysiology, NIMH	1322	6.24
Experimental Therapeutics, NINCDS	1251	5.91
Medical Neurology, NINCDS	1181	5.58
Clinical Neuroscience, NIMH	1140	5.38
Biophysics, NINCDS	1139	5.38
Neuropsychiatry, NIMH	1030	4.86
Cerebral Metabolism, NIMH	992	4.68
Psychology & Psychopathology, NIMH	987	4.66
Neural Control, NINCDS	909	4.29
Biological Psychiatry, NIMH	817	3.86
Neuropsychology, NIMH	765	3.61
Child Psychiatry, NIMH	702	3.31
Molecular Biology, NIMH	404	1.91
Neurochemistry, NINCDS	362	1.71
Cell Biology, NIMH	321	1.52
Molecular Biology, NINCDS	308	1.45
Molecular Genetics, NINCDS	301	1.42
Surgical Neurology, NINCDS	287	1.36
Neurobiology, NINCDS	269	1.27
Preclinical Pharmacology, NIMH	231	1.09
Clinical Science, NIMH	228	1.08
Clinical Neurosciences, NINCDS	152	0.72
Clinical Neurogenetics, NIMH	138	0.65
Neuropathology & Neuroanatomical Sciences, NINCDS	114	0.54
*NIMH (TOTAL)	12,066	56.97
*NINCDS (TOTAL)	7,830	36.97
*NICHD (TOTAL) **	1,284	6.06
	21,180	100.00

*These figures represent our total effort; they include time for labs not listed individually.

**NICHD loans the Section one position, and is thus entitled to 1700 hours of service.

TAB 2 -- LABORATORY OF BIOPHYSICS -- (LB)

ANNUAL REPORT

October 1, 1984 through September 30, 1985

Laboratory of Biophysics
National Institute of Neurological and Communicative Disorders and Stroke

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Annual Report
October 1, 1984 thru September 30, 1985
National Institute of Neurological and Communicative
Disorders and Stroke
Laboratory of Biophysics
William J. Adelman, Jr., PhD, Chief

INTRODUCTION

Research in the Laboratory of Biophysics (LB) is concerned with achieving an understanding of the molecular basis for the functioning of neuronal cells, tissues and systems. The laboratory has two units. The Woods Hole (WH), Massachusetts unit has two sections: Neural Membranes (NM) and Neural Systems (NS), both located at the Marine Biological Laboratory. The Bethesda unit is the Section on Molecular Biophysics, located in Bldg. 36 at NIH. 1985 marks the tenth year of continuous operation of the Woods Hole unit of LB.

LB has long been a leader in the study of membrane channels. This study has developed concepts of channel behavior which have provided an understanding of the mechanisms for generating nerve impulses, synaptic activity, and higher integrative behavior of nervous systems.

Biophysical methods are integrated with modern ultrastructural and biochemical techniques in order to investigate complicated neuronal mechanisms at fundamental levels. These interrelations are not strictly conceptual, as methods, techniques, equipment and personnel develop in parallel and become part of the force and direction of LB. There are close ties in this connection between the Woods Hole and Bethesda units of LB.

It is hoped that the following summary of highlights of LB's recent work point to the fruitfulness of this approach.

Section on Neural Membranes.

The Section on Neural Membranes uses electrical, chemical, optical, electron optical, mathematical and computer science techniques to investigate the function of neural cells and tissues at limits approaching molecular levels. Thus, molecular structures responsible for membrane ionic channel function and axoplasmic transport are sought. Model systems are constructed, tested and developed to simulate a variety of neuronal functions.

The Section has completed the first phase of its study of the sodium channel gating mechanism in the squid axon. Gating currents were elicited using voltage clamp sinusoidal forcing functions such that the activation gating kinetics were set in motion. Making use of Fourier analysis of non-linear gating current response and its harmonic content, sufficient data were obtained to construct a new over-determined model of the transition kinetics of the molecular processes involved in gating. Comparison with the recently determined amino acid sequenced primary molecular structure of the sodium channel has given confidence that this new kinetic scheme is compatible with the sodium channel molecular structure. Encouraged by this success, the Section has begun similar experiments on the sodium inactivation mechanism and the preliminary results appear very promising.

The Section's studies on ultrastructure have revealed a new set of values for Myxicola Schwann sheath dimensions suggesting that this periaxonal space is similar to that in squid axons and that periaxonal K^+ accumulation during activity in this space is also similar to that occurring in squid axons.

The effort by the Section in improving image processing techniques continues in both electron and light microscopy. Three-dimensional electron microscopy of thick sections using successive specimen tilts and advanced Fourier processing of images is proceeding well. The new technique of stereoscopic measurement of particle velocity in successively timed images in differential interference light microscopy of living axonal transport is now proving very useful.

Work on primary culture of squid neurons has been highly successful and these cultured neurons are now becoming available routinely for biophysical and physiological studies by LB and others. These cells have been identified as neurons by immunofluorescent assay with tetanus toxin. Somal diameters range from 5 to 40 µm and neurite processes are several mm long. Embryonic cells from two other molluscs, Hermissenda and Octopus also grow under the same conditions.

The Section has studied the effects of lidocaine derivatives on sodium and potassium channels. Blockade of both types of channels by lidocaine occurs on the inner surface of the membrane after the drug has crossed the lipid membrane. Sodium channels must be open for the drug to reach its blocking site, but potassium channels can be blocked in either the open or closed state. One quaternary lidocaine derivative, QX572, however, required an open potassium channel to block. Because of QX572's long length as compared to lidocaine, it is suggested that the length of a drug molecule determines whether or not a potassium channel must be open before blockade can occur.

The Section has studied the role of Ca in transmitter release and facilitation at the squid giant synapse by evaluating the effect of changes in the external concentration of Ca on the excitatory postsynaptic potential (EPSP). Transmitter release followed the fourth power of external Ca at low stimulus frequencies. However, at higher stimulus frequencies, which caused facilitation of the EPSP, transmitter release was found to be related to a lower power of the external Ca concentration. These results strongly suggest that the release site has four Ca receptors, each of which opens a "gate" and that all four gates must be open to activate transmitter release. In addition, it is proposed that the closure of these gates is slowed following Ca binding. Therefore, it appears that facilitation results from partial activation of transmitter release sites by calcium binding to from one to three Ca receptors.

Section on Neural Systems.

The Section on Neural Systems takes a multidisciplinary approach to the question of how information is stored during learning and how it is made available for later recall. The experimental psychology program uses associative learning paradigms to produce persistent behavioral changes in the nudibranch mollusc Hermissenda crassicornis as well as vertebrate species such as rabbits. Quantitative assessments are made of the animals' responses to the conditioned and unconditioned stimuli before and after classical conditioning paradigms. These assessments include precise dissection of generalized behavioral transformations into modification of individual muscular components of the behaviors. A full range of psychological manipulations have been used to clearly establish the sensitivity of the learning behavior to the exact temporal relationship of the stimuli which are associated during acquisition of the learning. Also of interest to the psychologists is the close linkage of the learning behavior to the specific stimuli associated and discriminative functions involving those stimuli not associated.

The Section's neurophysiology program is concerned first with the definition of those neural systems relevant to the learning capability. Multiple intracellular recordings from pre-and postsynaptic neurons have been employed within the visual, vestibular and chemosensory pathways of Hermissenda to establish a working knowledge of the critical neural systems and to describe how information flows in a stepwise fashion beginning with the sensory cells at the input, continuing through integrating cells, and finally to motor cells at the output. A similar approach is being taken with the rabbit hippocampus, and critical afferent and efferent pathways within this structure. Neurophysiological correlates are then obtained (again for both the mollusc and the rabbit) for conditioned (as well as a variety of control) animals. These neurophysiological correlates are recorded in intact animals, isolated nervous systems, and isolated neuronal membranes. Based on such correlates, electrophysiological sequences are constructed to trace the transformation of the information in electrical terms of the neural systems.

The Section's biophysics program measures persistent modification of specific ionic channels during and following the learning. In the past, a two-microelectrode voltage clamp was employed to characterize genetically specified membrane currents within identified neurons which were demonstrated to play a causal role in the acquisition and retention of associative learning. More recently the patch-clamp technique has made it possible to analyze these currents on a single channel level. This technique has been used in both the cell-attached and "inside-out" configurations to determine which subcellular biochemical processes (e.g., Ca^{2+}-dependent phosphorylation) are critical for regulating those ionic channels which change during learning. All of these biophysical approaches have also been applied to unequivocally demonstrate that it is in fact persistent modification of specific ionic channels which encode a learned association for later recall.

The biochemistry research effort of the Section seeks to uncover the molecular basis for the persistent ionic channel modifications shown to underlie associative learning (both in Hermissenda and the rabbit). A variety of biochemical and molecular biological methods are being brought to bear for this purpose. Microgel analysis of phosphorylation of individual neuronal proteins, for example, has revealed that Ca^{2+}-dependent phosphorylation of a specific low-molecular weight protein changes within certain neurons of conditioned animals but not those exposed to control paradigms. Exposure of neurons to prolonged depolarization, which simulates the integrated visual-vestibular network effects on identified neurons during conditioning, is also followed by long-lasting phosphorylation differences for particular low molecular weight proteins. Furthermore, a number of intracellular manipulations have provided support for the hypothesis that learning-induced modification of ionic channels involves Ca^{2+}-calmodulin-dependent and possible Ca^{2+} and lipid-dependent phosphorylation. Such manipulations include iontophoretic injection of Ca^{2+}-calmodulin-dependent protein kinase (Type II brain), inositol triphosphate, or phosphatase, or preincubation with C-kinase activators such as phorbol esters or OAG. Modern molecular biological techniques are also now making available for the Section's use monoclonal antibodies to enzymes (e.g., the Type II kinase) implicated in the learning process. Other antibodies (to phosphatase) may also prove helpful for our reconstruction of the biochemical and associated biophysical sequences which make biological records of memory possible.

The cellular anatomy aspect of the Section's programs contributes in several ways to the various levels of inquiry into the learning process already mentioned. Ultrastructural measurements of the cells and their synaptic interaction has provided further definition of the relevant neural systems. Activity-dependent uptake of radioactive labels within these systems has been monitored by autoradiographic methods. Morphometric techniques, together with serial sectioning and computerized reconstruction, may uncover structural manifestations of the biophysical and biochemical changes already shown for neurons within conditioned but not control animals. Differential absorption spectrophotometry allows intracellular localization of fluctuations of cytosolic Ca^{2+} as they occur during different phases of the learning process. Cytochemical identification of individual neurons has also implicated neurochemical means of amplifying the Ca^{2+}-dependent modulation of the channels during learning.

Finally, the developmental biologists within the Section have established laboratory strains of Hermissenda. Such strains permit assessment of how genetic and environmental factors may interact to determine individual differences in the ability of the animals to undergo associative learning.

Perhaps most important in all of these efforts is the accumulated evidence that a remarkable similarity exists between means of encoding learned associations in the snail and the rabbit. The same learning-induced reduction of well-characterized K^+ channels has been found to provide such encoding in Hermissenda as it does within identified neurons of rabbit hippocampal slices. Such parallel mechanisms may ultimately provide the basis for clinical intervention and thereby the amelioration of pathologic symptomatology.

Section on Molecular Biophysics

The interests of the Section have broadened in the past two years to include a wider range of membrane properties. Our interests now include the study of ionic channels in nerve, muscle, plants, and eggs and also the study of the mechanism of synaptic transmission. This broadening is based upon recent advances that have opened up additional possibilities for understanding membrane processes. The study of these several systems provides an opportunity for observing similarities and differences in membrane properties and thereby gaining additional insights.

Tissue-cultured cells have been used during the past year to study the effects of drugs on channels and to study possible interactions between channels. One study involves the role of diazepam in increasing the effectiveness of GABA in opening inhibitory postsynaptic channels. We have shown that a model wherein diazepam acts to lower the dissociation rate of GABA is consistent with the kinetic data. Work is continuing on testing whether an alternative theory - that diazepam acts directly to open channels - can be ruled out on the basis of the observed kinetics. This would be of clinical interest, since it would indicate a clear difference between the modes of action of diazepam and pentabarbitol, and would provide a rationale for the observation that overdoses of pentabarbitol are much more life-threatening than overdoses of diazepam. Pentobarbitol is known to act directly on the channel and, therefore, excessive doses can open too many channels and cause serious negative consequences. If diazepam acts only to decrease the dissociation of GABA, then even saturating concentrations of diazepam would have an effect limited to a finite increment in the affinity for GABA.

Another study on tissue-cultured cells addresses the question of possible interaction between individual sodium channels. This project involves a comparison between the behavior of patches of membrane with two sodium channels and the behavior of patches with one sodium channel. If two channels in a patch are equal and identical, then the probabilities that 0, 1 or 2 channels are open should follow a binomial distribution. We have found clear departures from this prediction, indicating that the channels are not equal or not independent or neither equal nor independent. An analysis is underway to obtain further insight into the two-channel system by determining whether or not the channels are independent. This is being accomplished by appropriate comparisons between the voltage dependence of the two-channel system and the voltage dependence found in one-channel experiments.

Theoretical and experimental studies are underway regarding the mechanisms involved at both the presynaptic and postsynaptic terminals. One study on the presynaptic terminal has recently been initiated in collaboration with the Section on Neural Membranes, and involves the possible role of increased osmolarity of vesicles on exocytosis of vesicular contents. Another study on the presynaptic terminal has been directed toward the effects of high oxygen tension. It has been found that 100% oxygen at atmospheric pressure enhances glutamate-mediated excitatory transmission and depresses GABA-mediated inhibitory transmission. Both of these effects are likely to be involved in oxygen-induced seizures that are observed clinically. Present studies are underway to elucidate the mechanisms for these effects of oxygen.

A theoretical study on the postsynaptic terminal involves analysis of the relative importance of the amplitudes and durations of individual contributions to the total postsynaptic potential. One of the purposes of this study is to calculate the clinical effectiveness of different types of synaptic drugs.

Work on the giant axon has addressed several aspects of channel behavior: the separation of activation and inactivation, the role of the Schwann-cell space, and the effects of phosphorylation. In the course of this work, the perplexing issue of the rising phase of gating current that is often seen experimentally has been resolved. It has now been shown that the rising phase is artifactual, and can be removed by reducing the series resistance of the Schwann-cell space.

Our previous work on patch-clamping wheat protoplasts has been extended to protoplasts of carrot roots and of pulvinus flexors and extensors. It is known that there is a movement of potassium to flexors in the evening and to extensors in the morning, and this has motivated us to use the patch-clamp method to search for potassium channels. We have recently found voltage-dependent potassium channels that can be blocked by tetraethylammonium ions, and work is continuing to determine whether these channels are influenced by light.

Our work on fertilization of sea urchin eggs has continued both experimentally and theoretically. These efforts have centered on the mechanism by which sperm triggers an increase in intracellular calcium in the egg. We have previously shown that injection into the egg of the soluble fraction of homogenized, centrifuged sperm triggers the increase in intracellular calcium. Current experimental work involves the determination of the active ingredient in the sperm. We have also developed a theoretical model for this process, based on the assumption that inositol triphosphate acts as an agonist to open calcium channels in internal organelles of the egg. The model predicts an important experimental feature of fertilization: the internal calcium concentration of the egg increases for a time, and then decreases. This model serves as a useful guide in our efforts at purification of the active factor in sperm.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 01950-14 LB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Excitable Membrane Characteristics: Voltage Clamp and Impedance Measurements.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
PI: W. J. Adelman, Jr. Chief LB, NINCDS

COOPERATING UNITS (if any)
University of Minnesota (J. Fohlmeister); Marine Biological Laboratory, Woods Hole, MA (C. Tyndale, R. Mueller, R. Waltz); Hamline University (J. Brennan); University of Maryland (R. French)

LAB/BRANCH
Laboratory of Biophysics, IRP

SECTION
Section on Neural Membranes (located at MBL, Woods Hole, MA 02543)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.6	0.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

While the anticonvulsants ethosuximide and valproate have widespread clinical use as antiepileptic drugs, their effect on primary excitable membrane processes has heretofore not been studied extensively. Using the squid giant axon as a model experimental system for the study of sodium and potassium channels, voltage clamp studies were carried out to test the effects of these agents on excitability. Both gating current and ionic currents were measured. Ethosuximide was shown to be an external voltage-independent sodium channel blocker, and was shown to have a potassium channel effect on both gating and open channel conductance. When applied internally both ethosuximide and valproate slow sodium channel gating, but valproate slows K channel gating without effect on the flux through open channels. These results suggest that both of these agents may have therapeutic properties directly at the membrane level that are unrelated to their synaptic effects.

Future work on this project will be incorporated into project Z01 NS 02087.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02087-12 LB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Function and Structure of Ionic Channels: Ion Interactions and Gating Mechanisms.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
PI: W. J. Adelman, Jr. Chief LB, NINCDS

COOPERATING UNITS (if any)
University of Minnesota (J. Fohlmeister); Marine Biological Laboratory, Woods Hole, MA (C. Tyndale, R. Mueller, R. Waltz).

LAB/BRANCH
Laboratory of Biophysics, IRP

SECTION
Section on Neural Membranes (located at MBL, Woods Hole, MA 02543)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.6	0.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Internally perfused protease-treated squid giant axons were prepared for gating current measurements. Gating current records obtained under large amplitude sinusoidal voltage clamp allowing for settling times into dynamic steady-states were analyzed as functions of the mean membrane potential of the test sinusoid for which the amplitude and frequency were held constant. Non-linear analysis measured the harmonic content (amplitudes and phases) of the distorted periodic current records. The most pronounced feature is a dominant second harmonic. This second harmonic is centered at E_{mean} = +10 mV. A number of other characteristic harmonic behaviors were also observed. The harmonics tend to die away for very negative (< -60 mV) and very positive (> +72 mV) values of E_{mean}. This harmonic behavior is basically different from that seen in gating current simulations of standard models, including the Hodgkin-Huxley model. The axonal data suggest two moving molecular components with independent degrees of freedom. On this basis, a new kinetic model of sodium activation gating was derived which differs from previous models in being over-determined by the data. The kinetics that simulate the experimental data contain two independently constrained molecular processes. The model predicts 1) sizable gating currents in response to hyperpolarizing voltage steps from rest, 2) a substantial increase in the initial peak of the gating current following voltage steps from prehyperpolarized potentials, 3) a small delay in the onset of sodium ion current following voltage steps from prehyperpolarized potentials, and 4) flickering during the open state in single channel current records. The present model reproduces the phenomenological development of Na conductance during the initiation and development of action potentials. A model gate based on this kinetic scheme and the primary amino acid structure of the sodium channel has been constructed. Future work on Project Z01 NS 01950 will be incorporated into this one.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02092-12 LB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Subcellular and Extracellular Structure Associated with Nerve and Muscle.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W. J. Adelman, Jr.	Chief	LB, NINCDS
Others:	P. Roslansky	Guest Researcher	LB, NINCDS

COOPERATING UNITS (if any) Marine Biological Laboratory, Woods Hole, MA (A. Hodge, R. Waltz, C. Tyndale, R. Mueller); Case Western Reserve (R. Lasek); Dartmouth College (R. Allen); University of Toronto (C. Govind); The Technion, Haifa, Israel (N. Moran, Y. Palti, E. Levitan)

LAB/BRANCH
Laboratory of Biophysics, IRP

SECTION
Section on Neural Membranes (located at MBL, Woods Hole, MA 02543)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.9	3.7	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to examine the subcellular and extracellular structure of nerve and muscle and relate such structure to function. Electron microscopy in TEM, STEM and analytical electron beam probe modes, such as EELS and EDAX, determination of proteins contributing to these structures and structural modeling are methods used in this study. The following structures are probed: 1) Neuroplasmic lattice, 2) neurofilaments, 3) microtubules, 4) axolemma, 5) glial cell membranes, and 6) myofilaments. Methods developed and used in this study are: 1) Stereoscopic imaging, 2) optical autocorrelation, 3) fast Fourier transformation (FFT) of STEM video images, and 4) STEM video image filtering and image enhancement using reverse Fourier transformation. Video imaged light microscopy is used to study living neurons in differential interference contrast. A new method was developed for direct visualization of particle velocity distribution. By viewing image pairs separated by an appropriate time interval in sequential recording of the subject, the positive or negative parallax arising from particle motion results in the binocular image of a particle being perceived as raised or lowered relative to an immobile background plane depending on its direction of movement. The degree of perceived elevation is proportional to particle speed. Using this method, measured particle movement during axoplasmic transport in squid axons ranged from 0.05 to 0.75 µm/sec. Morphological studies using electron microscopy of sections of Myxicola giant axons showed that the thickness of the Schwann cell layer is about 10 µm and the thickness of the periaxonal space is from 10-20 nm. These values were shown to be consistent with electrical studies of periaxonal K^+ accumulation using voltage clamp methods.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02606-02 LB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Chemical Transmission at the Squid Giant Synapse

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E. F. Stanley	Visiting Scientist	LB, NINCDS
Others:	W. J. Adelman, Jr.	Chief	LB, NINCDS

COOPERATING UNITS (if any)
Marine Biological Laboratory, Woods Hole, MA (C. Tyndale).

LAB/BRANCH
Laboratory of Biophysics, IRP

SECTION
Section on Neural Membranes (located at MBL, Woods Hole, MA 02543)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The role of Ca in transmitter release and facilitation has been examined at the squid giant synapse by evaluating the effect of changes in the external concentration of Ca on the excitatory postsynaptic potential (EPSP). Transmitter release followed the fourth power of external Ca at low stimulus frequencies. However, at higher stimulus frequencies, which caused facilitation of the EPSP, transmitter release was found to be related to a lower power of the external Ca concentration. It is proposed that the release site has four Ca receptors, each of which opens a "gate" and that all four gates must be open to activate transmitter release. In addition, it is proposed that after Ca binding the closure of the gates is slow and, hence, that facilitation is due to the partial activation of release sites by a subthreshold one to three Ca ions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02607-02 LB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Structure and Function of Tissue Cultured Invertebrate Neurons.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W. J. Adelman, Jr.	Chief	LB, NINCDS
Others:	R. V. Rice	IPA Fellow	LB, NINCDS

COOPERATING UNITS (if any)
Marine Biological Laboratory, Woods Hole, MA (J. Harrigan and R. Mueller); University of Hawaii (J. Arnold).

LAB/BRANCH
Laboratory of Biophysics, IRP

SECTION
Section on Neural Membranes (located at MBL, Woods Hole, MA 02543)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.7	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of this project is to culture neurons in the laboratory for use in studies of axoplasmic structure and transport. These cultured neurons are also to be used in connection with voltage clamp and patch clamp experiments of ionic channels and their conductances and gating mechanisms. The routine of culturing squid embryonic neurons has now been established. Eagle's media components dissolved in artificial sea water, 20% heat inactivated fetal bovine serum, and antibiotics when used with Primaria plastic dishes result in principally neurons (and some muscle cells) when incubated at 22°C. Squid embryonic stages from 18 to 26 give most reliable results when head regions are dissected free of yolk. Indirect immunofluorescent assay with tetanus toxin specific for neurons showed most cells to be neuronal. Cells live in culture for over a month. Cells grow after subculturing but do not persist probably for lack of nerve growth factor. Somal diameters range from 5 to 40 microns; neurite processes extend to millimeters; cell morphologies vary from bipolar to pyramidal with six or seven neurites. Embryonic cells from two other molluscs, Hermissenda and Octopus, also grow under the same conditions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	**PROJECT NUMBER** Z01 NS 02608-02 LB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Comparative Aspects of Ionic Conductances in Nerve and Heart Cell Membranes.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: J. R. Clay Physicist LB, NINCDS

COOPERATING UNITS *(if any)*
Marine Biological Laboratory, Woods Hole, MA (R. Mueller, C. Tyndale); McGill University (A. Shrier); University of Minnesota (M. Bacaner).

LAB/BRANCH
Laboratory of Biophysics, IRP

SECTION
Section on Neural Membranes (located at MBL, Woods Hole, MA 02543)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.3	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is concerned with a comparative analysis of ionic current channels in nerve and heart cell membranes with a particular emphasis on the effects of cardioactive drugs on both preparations. During the past year the primary experimental preparation which has been used has been the squid giant axon. The effects of lidocaine and its derivatives on the sodium ion current, I_{Na}, and the potassium ion current, I_K, have been studied. The mechanism by which lidocaine blocks I_K is different from the mechanism by which it blocks I_{Na}. Blockade of both types of channels occurs on the interior surface of the membrane, after the drug has crossed the lipid portion of the membrane. However, the sodium channel gates must open before a lidocaine molecule can reach its blocking site within the channel, whereas a potassium channel can be blocked regardless of whether or not its gates are open. This result suggests that the inner mouth of the potassium channel is accessible to drug molecules when the channel is either in its open or its closed state, whereas the inner mouth of the sodium channel is not accessible to drugs when the channel is in its closed state. This comparative analysis of I_K and I_{Na} is valid, in general, for most drugs. One exception is QX572, a quaternary derivative of lidocaine. The potassium channel gates must open, as is the case with the sodium channel, before blockade by this drug can occur. The QX572 molecule is about twice as long as the lidocaine molecule, which suggests that the length of a drug molecule determines whether or not a potassium channel must open before channel blockade can occur.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02151-11 LB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Information Processing in Simple Nervous Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D.L. Alkon	Medical Officer	LB NINCDS
Others:	C. Collin	Visiting Fellow	LB NINCDS
	J. Disterhoft	IPA Fellow	LB NINCDS
	R. Forman	Staff Fellow	LB NINCDS
	M. Kubota	Visiting Fellow	LB NINCDS
	A. Kuzirian	Staff Fellow	LB NINCDS
	S. Naito	Special Expert	LB NINCDS
	M. Sakakibara	Visiting Fellow	LB NINCDS

COOPERATING UNITS (if any)
Marine Biological Laboratory, Woods Hole, MA 02543 (J. Harrigan, I. Lederhendler, D. McPhie, J. Neary); Northeastern University (E. Meyhofer); Boston University Marine Program (C. Chen; D. Coulter)

LAB/BRANCH
Laboratory of Biophysics, IRP

SECTION
Section on Neural Systems (located at MBL, Woods Hole, MA 02543)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
9.0	8.5	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The principal objective is to study the mechanisms by which simple neural networks process information with particular emphasis on mechanisms of learning. The nervous system of Hermissenda crassicornis has proven to be a good model for information processing at several levels: sensory transduction by photoreceptors and hair cells, analysis of synaptic circuitry, changes in synaptic circuitry produced by conditioning paradigms administered to intact animals, as well as to isolated nervous systems, membrane properties modified by conditioning, identification of critical developmental stages for the neural networks of interest, as well as stages critical for learning. Techniques employed thus far to pursue these questions include simultaneous intracellular recording from multiple neural elements, paired stimulation of the visual and vestibular pathways using a rotating table, iontophoresis of fluorescent dyes and electron dense materials, electron microscopy, automated behavioral monitoring of intact Hermissenda, voltage clamp of identified neural elements. Other methods include mariculture, subcellular fractionation, protein phosphorylation analysis, uptake of neurotransmitter precursors, phosphoprotein characterization and purification, and immunologic protein identification. Patch clamp of membrane fragments of identified neurons is also being combined with enzymatic regulation of specific channels changed by learning to determine molecular mechanisms for encoding associatively learned information. Analogous protocols are also conducted with brain slices from neuronal aggregates which mediate classical conditioning of the rabbit nictitating membrane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02088-12 LB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Function and Structure of Membrane Ionic Channels

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	G. Ehrenstein	Research Physicist	LB NINCDS
Other:	N. Moran	Visiting Associate	LB NINCDS
	K. Iwasa	Senior Staff Fellow	LB NINCDS

COOPERATING UNITS (if any)

Weed Science Laboratory - AEQI, Dept. of Agriculture, Beltsville, MD.
(C. Baire and C. Mischke)

LAB/BRANCH

Laboratory of Biophysics, IRP

SECTION

Section on Molecular Biophysics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.3	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
  ☐ (a1) Minors
  ☐ (a2) Interviews
☐ (b) Human tissues
☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The BTX-modified sodium channel has been studied under patch clamp for cases where the patch contains only one channel and for cases where the patch contains two channels. The open probabilities of some two-channel pairs do not conform to a binomial distribution. This indicates that the oft-used assumptions that similar channels are equal and independent are not always valid.

Channels in plant protoplasts have also been studied under patch clamp. So far we have tested wheat, carrot roots, and pulvinus flexors and extensors. Channels have been observed in all of these cells. We have recently observed pulvinus potassium channels. Since potassium is known to move to the flexor in the evening and to the extensor in the morning, we are presently attempting to determine whether the potassium channels are sensitive to light.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02091-12 LB

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mathematical Modeling

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. FitzHugh	Research Physicist	LB NINCDS
Other:	G. Ehrenstein	Research Physicist	LB NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Biophysics, IRP

SECTION

Section on Molecular Biophysics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mathematical modeling of the following phenomena was done:

Signal detection and analysis of the square wave currents from single channels opening and closing in a membrane, distorted by noise and low-pass filtering.

The spread of the fertilization membrane, through release of calcium, over the surface of a spherical marine egg.

The release of transmitter at a squid giant synapse through the binding of free Ca^{++} ions produced by a presynaptic stimulus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02218-10 LB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Affect of Drugs on Voltage-Dependent Ionic Conductance in Membranes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.L. Gilbert	Research Physiologist	LB NINCDS
Other:	C. Colton	Special Expert	LB NINCDS

COOPERATING UNITS *(if any)*
R. J. Lipicky, Food and Drug Administration; E. Wenkert, Dept. of Chemistry, UCLA at San Diego; H. Pant, National Institute on Alcohol Abuse and Alcoholism, ADAMHA J. Colton, National Science Foundation.

LAB/BRANCH
Laboratory of Biophysics, IRP

SECTION
Section on Molecular Biophysics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	2.0	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to better understand how drugs affect the mechanisms of the ionic conductance in membranes which are voltage-dependent and excitable. Emphasis has recently focused on the effect of the reactive derivatives of molecular oxygen on the lobster neuromuscular junction. Oxygen and these derivatives have been implicated as causative agents in a wide number of physiological and pathological processes, such as the bactericidal action of granulocytes, aging, and ischemic reperfusion injury. We have found that 1 micromolar hydrogen peroxide inhibits reversibly the evoked excitatory junction potential (ejp). This effect is not on the glutmate post-synaptic receptor, since this peroxide inhibition is abolished when glutamate is exogeneously applied to the preparation. At concentrations above 0.1 millimolar hydrogen peroxide, the peroxide inhibition is only partially abolished in the presence of glutamate. Thus, at high concentrations, the post-synaptic receptor is inhibited. Pretreatment with thiourea, a free radical scavenger, provides some protection against the peroxide inhibition. This is evidence suggesting that at least part of the peroxide inhibition is mediated by the hydroxyl free radical. Part of the peroxide inhibition might also be due to hydroperoxide production and membrane lipid peroxidation. The effect of elevated partial pressures of oxygen on the squid giant synapse is also being investigated.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02317-08-LB

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Excitable Membranes and Ion Channels in Cultured Nerve and Muscle Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. H. Lecar	Research Physicist	LB NINCDS

COOPERATING UNITS *(if any)*

LN NINCDS; Tissue Transplantation Program Center, NMRI (S. Yeandle);

LAB/BRANCH

Laboratory of Biophysics

SECTION

Section on Molecular Biophysics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Single-channel currents are measured in isolated patches of excitable-cell membranes using the patch electrode method. Stochastic activation of gated ionic channels is studied as an indicator of the molecular conformation changes underlying excitation in the nervous system. Inhibitory postsynaptic channels from mouse spinal cord neurons and electrically activated potassium channels from neuroblastoma, myeloma and lymphocytes have been the main objects of study. Modification of channel gating by pharmacological agents and neurotransmitters is studied as a means of establishing a picture of synaptic integration based on the properties of membrane ionic channels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02526-04 LB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gated Ionic Channels in Membranes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

R. E. Taylor	Research Physiologist	LB NINCDS

COOPERATING UNITS (if any)

Dept. of Physiology, UCLA, Los Angeles, CA (F. Bezanilla, J.R. Stimers and R.M. Torres) Marine Biological Laboratory, Woods Hole, MA

LAB/BRANCH

Laboratory of Biophysics

SECTION

Section on Molecular Biophysics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
  ☐ (a1) Minors
  ☐ (a2) Interviews
☐ (b) Human tissues
☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are continuing the study of the effects of increased outside osmolarity. The results are impressive and we feel that they are due to improvement in the uniformity of the spatial control of the voltage clamp resulting from expansion of the space between the membrane of the axon and that of the Schwann cell. The results of analysis of a model for the effects of increasing the external osmolarity were presented at the International Biophysics Congress in Bristol, England in 1984.

We are in the process of completing the experimental work and writing two papers on the effects of changing the osmolarity of the external solution. One paper on the time domain aspect, i.e., the absence of a rising phase in the gating current or a slow component of the capacity transient and another in the frequency domain. With a new frequency domain program we can go to higher frequencies and have found another eigenvalue for the gating current in the neighborhood of 10 kHz.

In 1983 we were able to record sodium current fluctuations with good bandwidth using the cut-open axon and to extract functional channels and incorporate them into bilayers. This work will continue.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02609-02 LB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of Egg Activation Following Fertilization

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	G. Ehrenstein	Research Physicist	LB NINCDS

COOPERATING UNITS (if any)

Emory University, Atlanta, GA (L. DeFelice)
Stazione Zoologica, Naples, Italy (B. Dale)

LAB/BRANCH

Laboratory of Biophysics, IRP

SECTION

Section on Molecular Biophysics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethenda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have preveiously demonstrated that a fertilization membrane forms around a sea urchin egg when it is injected with a soluble spermatozoa fraction isosmotic with seawater. We have now performed additional control experiments to demonstrate that the active ingredient of sperm that triggers the formation of the fertilization membrane is not calcium. Experiments are in progress to purify the active ingredient of the soluble spermatozoa fraction.

TAB 3 -- LABORATORY OF CENTRAL NERVOUS SYSTEM STUDIES -- (CNSS)

ANNUAL REPORT

October 1, 1984 through September 30, 1985

Laboratory of Central Nervous System Studies

National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT
Laboratory of Central Nervous System Studies
October 1, 1984 — September 30, 1985

The major accomplishments in the past year of our laboratory have culminated in our planning, organizing and convening and conducting a first major workshop in which investigators in basic neurobiology in axonal transport together with the major world contributors to molecular structure of neurofilaments, neurotubules, paired helical filaments of neurofibrillary tangles, and cerebral amyloid met together for a long discussions on the implications of these findings for the pathogenesis of chronic degenerative brain disorders.

The conference was planned and organized by the LCNSS and sponsored by the Foundation pour l'Etude du Système Nerveux Central et Périphérique (FESN) on whose board the laboratory chief, D. Carleton Gajdusek, serves, and was held in Geneva on April 11-13, 1985. Five members of our team (P.W. Brown, D.C. Gajdusek, R.M. Garruto, R.G. Rohwer, and B.H. Toh) and many of our close collaborators met together with J. Axelrod, K. Beyreuther, A. Bignami, J. Blok, X. Breakfield, D. Dahl, A. Dahlstrom, H. Diringer, D. Grafstein, P. Hoffman, R.T. Johnson, C. Marotta, J. Martin, A. Matus, P. Merz, A.L. Notkins, T.S. Reece, D.J. Shelanski, and Y. Yase in an eminently successful intensive conference of three days of discussions lasting far into the night.

FESN WORKSHOP ON MOLECULAR MECHANISM OF PATHOGENESIS OF CNS DISEASE

The main purpose of the workshop was to convene investigators from different countries and with different backgrounds but sharing a common denominator, i.e., research interests that could be relevant to the understanding of degenerative, autoimmune and genetic diseases of the CNS, the main emphasis being on Alzheimer's senile dementia. Gajdusek's provocative hypothesis that many conditions as disparate as Alzhieimer's senile dementia and amyotrophic lateral sclerosis may share a disturbance of axonal transport as a common pathogenetic mechanism was the main reason for the first session devoted to this topic. The sessions on Alzheimer's disease and slow virus infections highlighted the major recent discoveries in these fields but left many unanswered questions, such as whether the small polypeptides with unique amino acid sequences forming brain amyloid in these conditions are themselves etiological agents, or, instead, a secondary phenomenon, products of a normal gene deregulated by viral infection or calcium-aluminum-silcate-protein complexes amenable to crystallographic analysis as to their mechanisms of growth. The session on immunopathogenesis highlighted the recently discovered role of astrocytes in brain inflammation and the effect of virus infections as deregulators of the immune system leading to autoimmune reactions. The application of molecular biology technology to genetic CNS disease was discussed in the last session. These methods have allowed the identification in human DNA of the abnormal gene responsible for Huntington's disease. Introduction of this gene in normal neurons grown in vitro may tell us that the gene is doing at the molecular level.

Intraneuronal accumulations of neurofilaments are a common finding in many degenerative neurological diseases including amyotrophic lateral sclerosis as well as in aluminum and beta,beta'-iminodipropionitril intoxication. Alzheimer's senile dementia, Guamanian parkinsonism with dementia and pugilistic encephalopathy are also characterized by intraneuronal accumulations of abnormal filaments in neurofibrillary tangles. They react like amyloid (birefringence after Congo red staining) and at the ultrastructural level they appear as tightly adherent pairs of helically-wound filaments. Alzheimer's senile dementia is also characterized byamyloid plaques: extracellular accumulations of amyloid fibrils within the cerebral cortex. Amyloid plaques are also commonly found in slow virus infections: kuru, Jakob-Creutzfeldt disease and scrapie. The workshop was opened by Gajdusek with a provocative hypothesis that interference with axonal transport of neurofilament may provide a common pathogenetic mechanism for such diverse conditions with etiologies varying from slow viruses to metal intoxication and with the suggestion that the larger keratinoid proteins of the triad forming the neurofilament (200,000, 145,000, and 70,000 dalton molecular weight) may be the precursors which are degraded into vascular amyloid in the CNS, amyloid in neuritic and amyloid plaques, and paired helical filaments in neurofibrillary tangles. The book covering this meeting is now in press and will appear early in 1986.

1. Interference with Axonal Transport of Neurofilament: The Underlying Mechanism of Pathogenesis in Alzheimer's Disease, Amyotrophic Lateral Sclerosis, and Many Other Degenerations of the CNS

Kuru and the transmissible virus dementias have been classified in a group of virus-induced slow infections that we have described as subacute spongiform virus encephalopathies because of the strikingly similar histopathological lesions they induce. Scrapie, mink encephalopathy, and the chronic wasting disease with spongiform encephalopathy of captive mule deer and of captive elk all appear, from their histopathology, pathogenesis, and the similarities of their infectious agents, to belong to the same group. The basic neurocytological lesions in all these diseases are a progressive vacuolation in the dendritic and axonal processes and cell bodies of neurons and, to lesser extent, in astrocytes and oligodendrocytes; an extensive astroglial hypertrophy and proliferation; and spongiform change or status spongiosis of gray matter. These atypical infections differ from other diseases of the human brain, which have been subsequently demonstrated to be slow virus infections, in that they do not evoke a virus-associated inflammatory response in the brain (i.e., no perivascular cuffing or invasion of the brain parenchyma with leucocytes); they usually show no pleocytosis nor do they show marked rise in protein in the cerebrospinal fluid throughout the course of infection. Furthermore, they show no evidence of an immune response to the causative virus and, unlike the situation in the other virus diseases, there are no recognizable virions in sections of the brain visualized by electron microscopy; instead, they show ultrastructural alteration in the plasma membrane that lines the vacuoles and piled up neurofilament in some swollen nerve cells.

The pursuit of the transmissibility and virus etiology of kuru and the presenile dementia of the Creutzfeldt-Jakob disease (CJD) type has led to the definition of the unconventional viruses as a new group of microbes which, because of their very atypical physical, chemical, and biological properties, has stimulated a world-wide quest to elucidate their structures and resolve the many paradoxes they present to the basic tenets of microbiology and to solve the enormous clinical and epidemiological problems these viruses pose. The unanticipated ramifications of the discovery of these slow infections and the peculiar properties of the unconventional viruses, which have even challenged the central dogma of modern molecular biology, have led to a series of discoveries each of which have wide implications to microbiological and neurobiological research. These are summarized below.

A. Interference with Axonal Transport of Neurofilament. A Newly Recognized Mechanism of Pathogenesis in Slow Virus Infection, Alzheimer's Disease, Amyotrophic Lateral Sclerosis and Many Other Degenerations of the CNS

The cytoskeleton of all cells contain three ultrastructurally distinct elements made of fibrous macromolecules: microtubules 24 nm in diameter, intermediate filaments 10 nm in diameter, and microfilaments about 5 nm in diameter and composed of polymerized actin.

Neurofilaments, also called neuronal intermediate filaments, are antigenically distince from the intermediate filaments of other cells. They extend from the cell body down the whole length of the axon; they are composed of three proteins of 200,000, 150,000, and 70,000 daltons molecular weights, respectively, and are usually associated with an additional 62,000 dalton protein. Our work on the etiology of kuru and on the cause of amyotrophic lateral sclerosis (ALS) and parkinsonism with dementia (PD) with the early appearance of neurofibrillary tangles (NFT) in the populations in high incidence foci in the western Pacific has led us to the realization that this molecular complex is not a static cytoskeletal structure, but a moving fiber, perhaps itself responsible for the slow component of axonal transport of lysosomes, enzymes, and transmitter molecules to the presynaptic terminals.

We now have evidence that suggests that interference with the transport of this 10 nm neurofilament complex may be responsible for formation of paired helical filaments (PHF) in the neurofibrillary tangles (NFT) and the neuritic plaques which characterize Alzheimer's disease. Furthermore, there are indications that amyloid deposits in the nervous system, particularly the amyloid plaques of Alzheimer's disease and those of Down's syndrome and Pick's disease and the perivascular accumulations of amyloid in the CNS, sometimes even in the vascular walls, may be also derived from neurofilament accumulations, while the paired helical filaments of NFT may represent yet further intracellular degradation of the protein triad from which 10 nm neurofilaments are formed.

The 4,000 dalton subunit protein of vascular amyloid, amyloid plaque cores, and also that of PHF from NFT of Alzheimer's disease all have the same amino acid sequence with progressively more N-terminal heterogeneity, respectively. This indicates that vascular amyloid deposits are least degraded from the parent host protein, core amyloid of amyloid plaques next, and the amyloid protein of PHF most degraded from this same parent protein specified by the host's genes. While protein components of microtubules (alpha and beta tubulin or MAPs proteins) might well be the precursor or parent protein we seek, we now find that in all conditions where these masses of amyloid appear (perivascular or in neuritic or amyloid plaques and NFTs) there is a pooling or piling up of neurofilament in perikaryon and axonal swellings. In fact, Hirano has demonstrated ultrastructurally minute masses of amyloid fibers and of regular paracrystalline arrays of particles or tubules within packed masses of piled up NF in spheroids, which have formed from such swollen perikaryons or axonal swellings in motor neurons of the spinal cord in amyotrophic lateral sclerosis.

Thus, interference with axonal transport of neurofilament may be a basic mechanism of pathogenesis that leads to 1) pooling of the neurofilament in the perikaryon or axonal cylinders and lysis of the neuron as in ALS and other motor neuron diseases; 2) amyloid plaque formation, from degradation of the same neurofilament proteins, in Alzheimer's disease and many other CNS degenerations; 3) Buninga, Hirano, and Lewy bodies and paracrystalline arrays found in many CNS degenerations; 4) neurofibrillary tangles and neuritic plaque formation with neurofilament further modified to form paired helical filaments; and finally, 5) amyloid deposition in the vascular intima or in the perivascular area as in congophilic angiopathy.

The larger, more regular amyloid plaques of kuru, of Creutzfeldt-Jakob disease (CJD) and its Gerstmann-Sträussler variant and of scrapie are also composed of an amyloid protein, presumably a degradation product of a host-specified large glycoprotein. The recent demonstration that this form of CJD human brain amyloid in plaque cores carries the same amino acid sequences as does the purified 28-30 kDa protein of scrapie associated fibrils (SAF) (or "prion protein") leads one to further conjecture about whether the same or different host protein(s) degenerate into these two types of CNS amyloid subunits; that of Alzheimer's and Pick's and Down's disease, and that of the atypical slow virus infections. Whether each type originates from different regions of the same host precursor protein or from different host proteins is not yet known. That of Alzheimer's disease and Down's syndrome is a self-aggregating 4 kDa amyloid protein, while that of CJD is a 7 kDa protein moiety which is heavily glycosylated to form a 28-30 kDa glycoprotein (approximately 20 kDa effective molecular weight) identical to the scrapie specific protein from scrapie associated fibrils (Prusiner calls this host specific protein his "prion" protein). What type of alteration is occurring to produce the regularly oriented configuration of beta-pleated sheets of birefringent amyloid proteins is not known. The known sequences of the amyloid in perivascular deposits, plaque cores and PHFs of neurofibrillary tangles, which are all alike, and the amino acid sequence of the SAF protein (or PrP) do not correspond. The precursor protein for amyloid formation in degenerative diseases of the brain (except for the slow virus infections) appears to be a component of neurofilament, but no sequence homology has yet been found between the sequences of these amyloids and those of the triad of proteins comprising neurofilament. For the different amyloids of the slow virus infections, all closely related to each other but not the amyloids of Alzheimer's disease, the precursor protein in normal cells has been identified as a 38 kDa protein. However, this host protein and its gene are not yet identified with a known function or structure in normal cells. The full sequence of the subunit proteins of normal neurofilament is not yet known, and thus the search for possible homology with neurofilament proteins cannot yet be completed.

B. Scrapie-associated fibrils

In suspension of scrapie-affected brain sedimented in a density gradient, Merz and Somerville have demonstrated an amyloid-like 2-stranded fiber—each fiber composed of two or four protofibrils--which increases in quantity with virus titer. We have found these structures in brains of CJD patients and in brains of primates with experimental CJD and kuru, but not in normal control brains or brains of patients with other neurodegenerative diseases. It has been postulated that these structures may represent the scrapie or CJD or kuru infectious agent. Such structures bring to mind the filamentous plant viruses and filamentous phage fd which are of about the same diameters. However, no nucleic acid has been demonstrated in purified preparations of SAF proteins (PrPs).

These scrapie associated fibrils which may be the infectious agents are distinguishable ultrastructurally from the paired helical filaments of neurofibrillary tangles and the fibrils of brain amyloid. However, their similarity is sufficient to demand close discrimination. Our discovery that the autoimmune antibody to 10 nm neurofilament which often appears in these slow virus diseases also reacts with the neurofibrillary tangles of Alzheimer's disease and the accumulations of 10 nm neurofilament in the brains of beta,beta'-iminodipropionitrile treated rats has lead us to the conjecture that the scrapie-associated fibrils may be related to normal 10 nm neurofilament, to the paired helical filament in neurofibrillary tangles, and to amyloid fibrils in the brain. Antisera, both polyclonal and monoclonal, to the PHFs of Alzheimer's disease NFTs crossreact with the purified subunit protein of amyloid from plaque cores of senile plaques. Some, but not all, antisera to normal neurofilament proteins crossreact with NFTs; these sera do not react with SAFs.

More recently, the western immunoblot technique used on the subunit proteins of the SAFs has shown that antibody to the 28-30 kDa subunit protein of SAFs (or Prusiner's "prion proteins", PrP) cross-react strongly with the subunit protein of SAFs from CJD and kuru affected brains. However, such SAF-specific sera do not react with neurofilaments or with PHF or plaque core amyloid from Alzheimer's disease. This scrapie-specific protein has been completely sequenced by, Multhaup, Diringer, Beyreuther and their groups; and the host gene specifying its precursor protein has been located using a synthesized 7-nucleic acid probe by Oesch et al. The DNA sequence of the host gene for the precursor protein has been determined for 250 amino acids (molecular weight 25 to 30 kDa). But the German group has found only 7,000 dalton molecular weight of protein in this scrapie specific protein (or prion protein); the rest is carbohydrate. One could predict that in such a carbohydrate-heavy glycoprotein that most antibodies to it would be directed to the carbohydrate moiety, and this appears to be the case. The polyclonal and monoclonal antibodies to the SAF protein fail to react with the deglycosylated 7,000 dalton polypeptide moiety.

C. Viruses Provoking No Immune Response and Evidencing no Non-Host Antigen

The CJD-kuru-scrapie-like slow viruses first invade the reticulo-endothelial cells and particularly low density lymphocytes in the spleen. Yet, they provoke no antibody response which can be demonstrated using live virus preparation of infectious titers over 10^{11} ID_{50}/gram. With the inability to demonstrate any anti-viral antibody response or any immune response directed against non-host viral components or capable of neutralizing the virus activity, these unconventional viruses become unique in their immunological behavior in microbiology. Natural and experimental infection with these viruses elicit no antibody response in the host nor does immunosuppression with whole body radiation, cortisone, anti-leukocytic serum or cytotoxic drugs alter the incubation period, progress or pattern of disease, or duration of illness to death. Finally, in vivo and in vitro study of both B-cell and T-cell function revealed no abnormality early or late in the course of illness and on in vitro no sensitization of the cells taken from diseased animals to high titer preparations of these viruses. Since high titer infective material in both crude suspension and highly purified also fail to elicit an immunological response against non-host components, even when used with adjuvants, this becomes the first group of microbes in which such immunological inertness has been demonstrated, and has evoked the speculation that the replication of these viruses does not involve production of a virus-specified non-host antigen.

Instead, their protein component may be specified by host genes and thus be recognized as self.

The soluble 28,000-30,000 dalton protein obtained from highly purified preparation of SAFs (prion protein, PrP) is noninfectious and is a subunit of the SAFs which apparently represents a fibrillary aggregation of such subunits. It appears to aggregate into dimers, tetramers, and hexadecamer polymers as does the subunit protein (7 kDa) of amyloid and PHFs. Antibody to this same scrapie protein has been made in rabbits and such polyclonal antibody reacts well with SAFs by an ELISA test and gold bead decoration immunoelectronmicroscopy. Such antibody to the scrapie SAFs cross-react well with the SAFs of kuru and of CJD and the Gerstmann-Sträussler form of CJD and already provides a quick means of diagnosis of these diseases.

Enormous resistance to physical and chemical inactivation.

The demonstration of the resistance of the unconventional viruses to high concentrations of formaldehyde or glutaraldehyde and most other antiviral and antiseptic substances, to ultraviolet and ionizing radiation, to ultrasonication, and to heat, and the further demonstration of iatrogenic transmission through implanted surgical electrodes, contaminated surgical instruments, and corneal transplantation, injections of human growth hormone derived from pituitary glands obtained from cadavers, and possibly through dentistry, has led to the necessity of changing autopsy room and operating theater techniques throughout the world as well as the precautions used in handling older and demented patients. Many of the gentle organic disinfectants, including detergents and the quarternary ammonium salts, often used for disinfection, and even hydrogen peroxide, formaldehyde, ether, chloroform, iodine, phenol and acetone, are inadequate for sterilization of the unconventional viruses as is the use of the ethylene oxide sterilizer. This demands revision of previously acceptable procedures for decontamination and disinfection.

These unconventional viruses are also resistant, even when partially purified, to all nucleases, to beta-propiolactone, ethylenediaminetetraacetic acid (EDTA), and sodium deoxycholate. They are moderately sensitive to most membrane-disrupting agents in high concentration such as phenol (60 percent), chloroform, ether, urea (6M), periodate (0.01M), 2-chloroethanol, alcoholic iodine, acetone, chloroform-butanol, to hypochlorite, to chaotropic ions such as thiocyanate and guanadinium and trichloroacetate, and to proteinase K and trypsin when partially purified, but these only inactivated 99 to 99.9% of the infectious particles, leaving behind highly resistant infectivity. Sodium hydroxide (1.0 N) and hypochlorite (5%), however, quickly inactivate over 10^5 ID_{50} of the virus. They have a UV inactivation action spectrum with a six-fold increased sensitivity at 237 nm over that at 254 nm or 280 nm, and 50-fold increased sensitivity at 220 nm. Moreover, they show remarkable resistance to ionizing radiation that indicates a target size, if such naive calculation is applicable to a highly aggregated "semisolid" arrays of associated proteins, of under 100,000 daltons.

However, many investigators have seen regular arrays of particles which appear to be tubular structures seen in cross section in presynaptic terminals of neurons in experimental animals infected with CJD, kuru and scrapie. Structures more typical of virions are not recognized on electron microscopic study of infected cells *in vivo* or *in vitro*, nor are they recognized in highly infectious preparations of virus concentrated by density-gradient banding in the zonal rotor.

These atypical properties have led to the speculation that the infectious agents lack a nucleic acid, and that they may be a self-replicating protein (perhaps by derepressing cellular DNA bearing information for their own synthesis), even a self-replicating membrane fragment which serves as a template for laying down abnormal plasma membrane, including itself. Gajdusek often suggested that they are templates catalyzing and organizing the specific degradation of a host-specified precursor protein, autocatalytically producing themselves in the process.

Analogies with defective or "contaminated" seed crystals of simple molecules specifying the crystallization of their own distinct crystal structure come to mind. The presence of mineral deposits in neurons in the form of hydroxyapatites often containing aluminum, silicon and other atoms as the antecedents to NFT formation with the amyloid protein of PHFs have been shown in the high incidence foci of amyotrophic lateral sclerosis, parkinsonism-dementia, and associated early appearance of NFTs in the Western Pacific. More recently, Masters et al. and Candy et al have found silicon and aluminum deposits in the center of amyloid plaque cores in Alzheimer's disease. Aluminum silicate, perhaps in the form of montmorillonites, is in the center of amyloid plaque cores. Candy et al. have suggested because of this location that they are the initiating elements of the amyloid deposition. We wonder whether a nucleus of a cation-binding mineral lattice may initiate the change to amyloid configuration of some keratinoid type host protein.

Mendelian single gene autosomal dominant inheritance determines expression in familial CJD.

CJD became the first human infectious disease in which a single gene was demonstrated to control susceptibility and occurrence of the disease. CJD virus is isolated from the brain of such familial cases. The autosomal dominant behavior of the disease in such families, including the appearance of the disease in 50% of siblings who survive to the age at which the disease usually appears, has evoked the possibility of virus etiology in other familial dementias. The presence of CJD patients in the families of well-known familial Alzheimer's disease, and the familial occurrence of the spino-cerebellar ataxic form of Creutzfeldt-Jakob disease, the Gerstmann-Sträussler syndrome, which is also transmissible, have led to renewed interest in familial dementias of all types. Techniques such as used by Gazella for locating the gene of Huntington's disease may now be used.

Auto-immune antibody to 10 nm neurofilament in SSVE patients.

The demonstration by Sotelo et al of a very specific auto-immune antibody directed against 10 nm neurofilaments and no other component of the CNS in over 60% of the patients with kuru and CJD, as a pheonomenon appearing late in the disease, was the first demonstration of an immune phenomenon in the SSVEs and an exciting new avenue of approach for the study of the transmissible dementias. This auto-immune antibody behaves like many other auto-immune antibodies such as the rheumatoid factor and the anti-DNA antibody in lupus and the anti-thyroglobulin antibody in Hashimoto's thyroiditis in that it is often present in normal subjects, and more often present in subjects closely related to the patients. Although found in more than half of patients with transmissible virus dementia, it was not detected in 40% of patients with classical CJD. It does develop in other gray matter diseases, including Alzheimer's and Parkinson's diseases, but at far lower incidence than in CJD.

Furthermore, it was not detected in patients with other immune diseases such as disseminated lupus erythematosis and chronic rheumatoid arthritis. We have demonstrated that on Western blots separating the three proteins comprising the 10nm neurofilament triad of 200 kDa, 150 kDa, 70 kDa most sera has antibodies directed against the 200 kDa protein with some cross reaction with the 150 kDa protein. Some sera react better with the 150 kDa protein, and rare sera only with the 70 kDa protein, thought to be an internal component of the neurofilament. Sheep with scrapie, however, often react best with a 62 kDa neurofilament associated protein. Some authors found a higher incidence of these specific antibodies in normal subjects than we have. Nonetheless, the same problem is posed. Why are there antibodies to the NF proteins and not to other CNS antigens?

Unconventional viruses: subviral pathogens, perhaps devoid of a nucleic acid or a non-host protein.

The scrapie virus has been partially purified by density-gradient sedimentation in the presence of specific detergents. Rohwer has succeeded in a 1000-fold purification of scrapie virus relative to other quantifiable proteins in the original brain suspension. In such preparations the virus is susceptible to proteinase K and trypsin digestion but it is not inactivated by any nuclease. Sedimented, washed and resuspended virus has been banded into peaks of high infectivity with the use of cesium chloride, sucrose, and metrizamide density gradients in the ultracentrifuge. Sucrose-saline density-gradient banding of scrapie virus in mouse brains produced wide peaks of scrapie infectivity at densities of 1.14 to 1.23 g/cm^3 . Attempts to demonstrate a non-host nucleic acid in scrapie virus preparations using DNA homology and transfection and nuclease inactivation have been unsuccessful. No significant quantities of nucleic acid are present in purified preparations of 28-30 kDa SAF associated protein (PrP protein), and such preparations are not infectious.

The atypical action spectrum for inactivation of scrapie virus by UV should not be taken as proof that no genetic information exists in the scrapie virus as nucleic acid molecules, since Latarjet has demonstrated similar resistance to ultraviolet and a similar UV action spectrum for microsomes. Ultraviolet resistance also depends greatly on small RNA size, as has been shown by the high resistance of the purified, very small, tobacco ring spot satellite virus RNA (about 80 kDa).

On the other hand, the unconventional viruses possess numerous properties in which they resemble classical viruses, and some of these properties suggest far more complex genetic interaction between virus and host than one might expect for genomes with a molecular weight of only 10^5 kDa. Rohwer has shown that the scrapie virus replicates in hamster brain at a constant rate, with no eclipse phase, and with a doubling time of 5.2 days. Examination of the kinetics of its inactivation and the demonstrated association or aggregation of scrapie virus particles into polymers or clusters which can be disrupted by ultrasonication have cast doubt on the calculation of its small size from ionizing radiation inactivation data and inferences about its structure from resistance to chemical inactivating agents. Thus, aggregates make necessary "multiple hits" for inactivation, while free virus is killed by a single event.

In plant virology we have recently been forced to modify our concepts of a virus to include subviral pathogens such as the newly described viroids causing eleven natural plant diseases (potato spindle tuber disease, chrysanthemum stunt disease, citrus exocortis disease, Cadang-Cadang disease of coconut palms, cherry chloratic mottle, cucumber pale fruit disease, hop stunt disease, avocado sunblotch disease, tomato bunchy top disease, tomato "planta macho" disease, and burdock stunt disease) and the virusoids of four natural plant diseases (velvet tobacco mottle virus, solanum nodiflorum mottle virus, lucerne transient streak virus, subterranean clover mottle virus) to which we may turn for analogy. All of the viroids are small circular RNAs containing no structural protein or membrane and they have all been fully sequenced and their fine structures determined. They have only partial base pairing as the circle collapses on itself. They contain only 246 to 574 ribonucleotides and replicate by a "rolling circle" copying of their RNA sequences in many sequential rotations to produce an oligomeric copy which is then cut into monomers or some times dimers. No protein is synthesized from their genetic information and only the replication machinery of the cell is used. These subviral pathogens have caused us to give much thought to possible similarities to the unconventional viruses. However, we and others have shown that the unconventional viruses differ markedly from the plant viroids on many counts.

Thus the intellectually stimulating analogies of the unconventional viruses to viroids and virusoids prove to be spurious, yet these subviral pathogens of plants have served to alert us to the possibility of extreme departure from conventional virus structures.

The delta antigen of infectious hepatitis, a defective replicating particle with only 1,700 bases on its genome (68,000 daltons) and requiring the infectious hepatitis B virus for its replication, offers further intriguing analogies to the unconventional virus.

Concluding Hypothesis--Fantasy of a "virus" from the inorganic world

We are at an exciting moment in the study of the unconventional viruses. Either a polymer of the SAF-associated protein (PrP protein) is the infectious agent directing its own synthesis by augmentation (and perhaps mistranslation) of its host gene, or this protein is simply an elegant molecular biological high-tech demonstration of what we have known for a long while; namely, that amyloid is found in the CNS in all of these diseases. In that case we are still in quest for the atypical virus.

If the formation of the self-polymerizing 28-30 kDa amyloid-like scrapie glycoprotein from a host protein by post-translational processing, peptide bond hydrolysis, cleavage, altered splicing and repacking is the basic growth process of scrapie replication, then the hydroxyapatite-aluminum silicate inorganic nidi in NFTs and in the center of amyloid plaque cores may signal that this mineral-protein complex is the replicating agent which has proved so elusive. We must allow for the possibility that such a mineral-amyloid complex might, in the proper milieu of the interior of a cell, replicate slowly and regularly as it degraded a 38 kDa host precursor protein to the amyloid we see in SAFs (PrP) and the amyloid plaques of these infections. In Alzhemer's and Pick's diseases and Down's syndrome a 4000 kDa polylpeptide or its polymers complexed as an amyloid protein to a calcium-aluminum-silicate apparently can self replicate and self aggregate as it autocatalytically degrades the precursor protein of neurofilament to the mineral-amyloid aggregates or paracrystalline arrays we see in neurofibrillary tangles and the amyloid plaque cores. It is an inference based on accumulating evidence, cited above, that the precursor protein is a component of intermediate filaments of the cytoskeleton. Only in the non-dividing neuron does this slow degenerative process eventually kill the cell. Thus, in the end, our atypical slow "virus" may simply be similar to a crystal template directing its own crystallization or "crystal lattice" from a source of presynthesized host protein precursors and an inorganic cation receptor nucleus. This remains a tenable hypothesis. If so, we wonder whether inorganic chemistry and crystallography may provide better insights than the normal paradigms of modern molecular biology.

POTENTIAL "EPIDEMIC" OF CJD FROM CONTAMINATION OF HUMAN GROWTH HORMONE OF PITUTARY ORIGIN

In 1984-85, within a period of a few months, 3 young adults who had been treated with human growth hormone (HGH) died in the United States with CJD, confirmed neuropathologically in 2 patients, and clinically compatible but as yet unconfirmed in 1 patient; an additional neuropathologically confirmed case has been identified in Great Britain.

The age-specific mortality rate for CJD in the population segment under 40 years of age is 0.01 case per million. Since approximately 10,000 Americans, all under age 40, have received HGH, the expected incidence of CJD in this group is 0.0001 case per year. Thus, the abrupt appearance of 3 cases of CJD in Americans under the age of 40 who had all been treated with growth hormone derived from pools of autopsied human pituitary glands, strongly incriminates CJD-contaminated growth hormone as the cause of disease.

From the available data, and allowing for the influence of some less well defined variables, an estimate can be made of the risk of inadvertent contamination of HGH by CJD virus. The U.S. annual mortality rate from all causes during the 1960-1980 period was approximately 0.9%, or, in the U.S. population of 250 million, somewhat fewer than 2.5 million deaths each year. Since the annual mortality rate of CJD is approximately 0.7-1.0 per million, or, in the U.S. population, somewhat fewer than 250 deaths per year, it follows that roughly 1 in 10,000 U.S. deaths is due to CJD. Because lots of pituitaries used in the preparation of HGH have varied from 500 to nearly 20,000 glands, frequent episodes of contamination could be expected to have occurred.

From numerous experimental transmission studies in which multiple animals have received aliquots of a single CJD-infected brain tissue suspension, it is known that after incubation periods of several years (depending on the species), clinical onsets tend to occur in a 'burst' at the center of a narrow bell-shaped curve, with a few scattered shorter or very much longer peripheral points. The recent 'cluster' of CJD cases following HGH therapy could thus represent the initial scattered short incubation period points in what will soon become an avalanche of iatrogenic CJD; or they may represent the burst itself, in which event few if any additional patients will be identified.

Epidemiologic studies already in progress will eventually determine if other recipients of HGH have been or will be affected by CJD, by identifying all CJD patients under the age of 40 dying in the United States during the past 10 years, and by identifying and following all HGH recipients.

The 3 U.S. patients had been exposed to a total of 33 different lots of HGH during therapy; at least 9 of these lots were shared by patients J.R. and P.G., and 1 other lot by patients J.R. and W.T., but no lot was shared by all 3 patients. Aliquots of the 26 still available lots to which these patients were exposed (including all of the shared lots) have already been inoculated into chimpanzees and squirrel monkeys (19 of the 22 lots given to patient J.R., all 15 lots given to patient P.G., and 2 of the 6 lots given to patient W.T.). Fifty-three additional lots dating as far back as the middle 1960's, that were not received by these patients, have also been inoculated into squirrel monkeys.

Arrangements are underway for similar inoculation experiments to be performed on all still available lots received by the British case, none of which overlapped with any lots given to the U.S. patients. Positive results will require minimum incubation periods of one year in the chimpanzee and two years in the squirrel monkeys, with the possibility of incubation periods of several years in the event of very low infectivity levels.

Immunoblot and electron microscopic analysis of purified brain tissue from patient P.G. has confirmed the diagnosis of CJD by the finding of scrapie-associated fibrils (SAF) and the associated 27 kDa marker protein specifically associated with the subacute spongiform virus encephalopathies. Similar analysis of brain tissue from the growth hormone recipient with a provisional diagnosis of amyotrophic lateral sclerosis has failed to detect either SAF or marker protein, confirming the clinical impression that this patient did not have CJD. All still available older lots, and selected newer lots, of HGH administered under National Pituitary Agency protocols are currently being tested by immunoblot for the marker protein. Although the assay is much less sensitive than animal transmission experiments, it has the enormous advantage of giving immediate results. Sera from patients who have received HGH therapy are being tested for the presence of antibody to this same protein, since these patients may have been immunized to any contaminating CJD protein during the course of their multiple intra-muscular HGH inoculations.

Experimental assessment of the level of infectivity likely to be present in lots of HGH already produced is also in progress. Manufacturing protocols used to produce HGH are being duplicated with the inclusion of known amounts of CJD virus, and the end product inoculated into thousands of experimental animals; CJD-contaminated HGH with the addition of ultrafiltration and sodium hydroxide exposure steps are being similarly inoculated in an effort to develop a product that, if needed, would be safe for future use.

PRIMATE MODELS OF ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS) WITH ISOLATION OF LENTIVIRUSES IN CHIMPANZEES

Human T-lymphotropic retroviruses are the cause of acquired immune deficiency syndrome (AIDS). To elucidate the role of these retroviruses in the pathogenesis of AIDS and in an attempt to develop an animal model for this disorder we inoculated 36 chimpanzees with brain and other tissues from AIDS patients or supernatant fluids from cell tissue cultures infected with HTLV-III, LAV, and IDAV-2. To date we have isolated AIDS-associated retrovirus from packed leukocytes from two chimpanzees inoculated intracerebrally and intravenously with brain tissue suspension. In addition, two chimpanzees inoculated with brain tissue and three chimpanzees inoculated with plasma or pooled visceral suspension have seroconverted to HTLV-III/LAV. Eleven chimpanzees inoculated with supernatant fluids from tissue cultures infected with HTLV-III (6), LAV (3) or IDAV-2 (2) have seroconverted to viral antigens between four and eight weeks after inoculation. Following primary inoculation, virus was recovered from each of six HTLV-III-inoculated chimpanzees and one of three LAV-inoculated chimpanzees. Second and third serial passages were accomplished using whole blood or tissue specimens from chimpanzees that had seroconverted to HTLV-III or LAV confirming active persistent infection. To date five chimpanzees have developed elevated absolute lymphocyte counts over $7000/mm^3$, and two chimpanzees have shown marked suppression of T-cell mitogen responses. None of the chimpanzees developed lymphadenopathy, opportunistic infection, tumors or encephalopathy after observation for 16 to 30 months. This report confirmed active and persistent virus infection of chimpanzees with retrovirus derived from blood of AIDS patients. In addition it established the presence of these retroviruses in the brains of patients with AIDS encephalopathy by direct transmission to chimpanzees.

Acquired immune deficiency syndrome has been under investigation for several years as a continuation of Dr. Gajdusek's investigations of a similar "AIDS" epidemic of interstitial plasma cell pneumonia in infants in Europe in the 1940s, '50s, and '60s which resulted in the first paper in English on *Pneumocystis carinii*. Both *P. carinii* and cytomegalic inclusion disease were causes of death; the epidemic receded without the primary cause of the immune deficiency having been identified.

PRIMARY LENTIVIRUS ENCEPHALOPATHY AS A MAJOR COMPLICATION OF AIDS

In collaboration with Dr. Leon Epstein at the University of Medicine and Dentistry of New Jersey the Laboratory of Central Nervous System Studies has been intensively investigating the role of human T-lymphotropic retrovirus in the pathogenesis of AIDS encephalopathy. The first demonstration of this retrovirus in human brain was accomplished by the transmission of infection to chimpanzees using brain tissue from AIDS patients with documentation of seroconversion and virus isolation in these animals. Subsequently, in collaboration with investigators at the NCI specific nucleic acid sequences of the human T-lymphotrophic retroviruses were identified within the brain of children and adults with AIDS encephalopathy.

Further neuropathological studies by Dr. Epstein and colleagues have resulted in the description of unique inflammatory cell infiltrates and multinucleated giant cells which are characteristic of primary retroviral encephalitis. Additional ultrastructural studies have demonstrated retroviral (lentiviral) virions within multinucleated cells and astrocytes.

The demonstration that the human T-lymphotrophic retroviruses are neurotropic as well as lymphotrophic has resulted in the classification of these retroviruses in the lentiviral subfamily.

These findings establish that a primary lentiviral encephalitis is part of systemic infection with the human T-lymphotropic retrovirus/lentivirus. This primary and persistent brain infection has profound clinical implications for the estimated two million individuals in the United States infected with this virus, and poses a major obsticlein the treatment of this infection.

HANTAAN VIRUS INFECTION IN THE USA AND WORLDWIDE (HEMORRHAGIC FEVER WITH RENAL SYNDROME

We continue to define the world wide problem of of hemorrhagic fever with renal syndrome (HFRS) which we have renamed *muroid virus nephropathy*, a viral zoonosis caused by a new group of Bunyaviruses. Previously our laboratory demonstrated that HFRS was the most important zoonosis and one of the most important virus diseases of all provinces of China and was caused by the same virus as that in Japan, Korea, and the Far Eastern Siberian USSR. In the past year we have demonstrated that Hantaan-like viruses are a Hantaan-related and is present in urban rats of most American cities. In addition, we have isolated Prospect Hill virus from meadow voles (*Microtus pennsylvanicus*) captured in Frederick, Maryland. The further clinical, virological and epidemiological elucidation of this world wide problem and the extension of it to the Americas will occupy dozens of laboratories for the next several decades.

The Prospect Hill virus has yielded a nephropathy model with proteinurea, and nitrogen retention in inoculated chimpanzees and cynomolgus monkeys; many other species of monkeys have been inoculated to determine their susceptibility. This is the first nephropathy model of a Hanvirus of the Bunabunyaviridae. Prospect Hill virus has had th three single-stranded RNA segments of its genome sequenced at the 3'-OH terminal for 15 to 20 nucleotides. It has thus proved to be a classical member of the Hanvirus group. We have also adapted the virus of nephropathica epidemica (Puumala virus) to cell culture and are passaging it serially in Mongolian gerbils (Meriones unguiculatus) as well as in laboratory-bred (Clethronomys glareolus).

SLOW UNCONVENTIONAL VIRUS INFECTIONS

In work on kuru, our most significant new contribution has been the clear documentation of incubation periods of thirty years and more in human kuru and the identification of the contaminating episode for several dozen patients occurring in recent years. We discovered that the great majority, in fact over ninety percent, of the infants and children of women present at a contaminating event of cannibalism have already come down with kuru. Continued surveillance has revealed no alteration in the pattern of kuru, the disappearance of which emphasizes the artifical man-made nature of the epidemic; kuru virus clearly has no reservoir in nature and no intermediate natural biological cycle for it preservation except in humans.

On Creutzfeldt-Jakob disease, our continued epidemiological work has made it clear that the one per million per annum incidence and death rate is approximately the same on all six continents in all nations and that high incidence foci are a real phenomenon. We have further demonstrated that in familial cases a single autosomal-dominant gene pattern of occurrence is indeed true in spite of the fact that the disease is caused by a virus. This is the first example in man of an autosomal-dominant single-gene inheritance controlling the appearance of an infectious disease.

The enormous resistance of the unconventional viruses causing kuru and Creutzfeldt-Jakob disease of man and scrapie in animals has resulted in altered procedures in all autopsy rooms, surgical theaters and clinics in the world. Our continued study of the inactivation and the physical properties of these agents is thus mandatory in order to set the proper standards for handling possible contamination.

The problem this resistance to inactivation may cause has reached enormous proportions with respect to the hepatitis B vaccine prepared from the hepatitis antigen in serum of human volunteers; some of these volunteers may be incubating the Creutzfeldt-Jakob dementia syndrome. Once this has been suggested, it is apparent that there is no assay procedure sufficient to declare the vaccine safe. Even a chimpanzee assay would require decades and still be uncertain, as shown by our newer work on variation in host range of human strains of Creutzfeldt-Jakob disease.

Our work with primates shows that peripheral routes of inoculation give irregular "takes" and, as expected, are associated with long incubation periods of perhaps one or more decades. We pointed out that an accident with this type of virus actually resulted in tens of thousands of cases of fatal scrapie in British sheep previously free of the disease when a formalinized louping-ill vaccine was contaminated with the scrapie virus. The moral, ethical and legal aspects of continuing to use the hepatitis B vaccine once this problem has been raised and appreciated are enormous.

Determining physical-chemical structure of the unconventional viruses using both a mouse-adapted strain of CJD virus and hamster and mouse strains of scrapie virus has been the major target of our laboratory. Recent highly-publicized speculations on the possible very exotic nature of these viruses are based in large degree on our data. Those speculations are ideas we have voiced over many years, but they are all still unprovable. Our own recent data again confirm the absence of any immune response to purified, high-titer virus or any involvement of the immune system in patients with the natural diseases or animals with experimental diseases. We have also been unable to demonstrate a nucleic acid by transfection and annealing (hybridization) techniques. By ultrasonication studies we found the high level of association of the hydrophobic viral particles into aggregates of 1000 monomers or more; this finding invalidates most of the studies in which an extremely small size has been determined by physical means, including equilibrium sedimentation, and also invalidates conventional interpretations of radiation resistance and chemical and enzyme resistances as well. On the other hand, it is clear that a new group of microbes has been defined that challenge the basic tenets of microbiology. Exotic new possibilities suggested by the scrapie virus include abnormal templates for laying down of plasma membranes and neurofilament, small proteins free of nucleic acids which are derepressors of cellular genes responsible for their own synthesis, or the first example of a filamentous virus in mammals. As a major problem for basic medical science, the resolution of this enigma is an inescapable challenge. Our most recent observation of unique helical fibrils in extracts of brains and spleens of animals with scrapie, kuru, and Creutzfeldt-Jakob disease, but not in controls, opens a new and promising possibility that the pathogenic agents themselves have finally been recognized and are a new form of pathogens--"filamentous viruses".

EPIDEMIOLOGY OF CJD IN ITS HIGH INCIDENCE IN THE MEDITERRANEAN BASIN

Our epidemiological studies of scrapie in France and elsewhere have reveal d that scrapie virus is nearly ubiquitous in butcher shops and restaurants of the world. That it may be responsible for occasional disease in primates has not been epidemiologically established. Yet we now know from our own inoculations that the human viruses of CJD or kuru can cause scrapie in goats, and that goat, sheep and mouse strains of scrapie can cause the Creutzfeldt-Jakob syndrome in several species of monkeys inoculated but not yet in chimpanzees. We have participated in the study of the transmissible scrapie-like agent affecting wild mule deer and moose in Colorado, and in the enormously intriguing demonstration that such infected mule deer develop amyloid plaques in great profusion, as do kuru victims and a portion of the CJD patients.

HIGH INCIDENCE FOCI OF ALS/PD AND EARLY APPEARANCE OF NFT'S IN WESTERN PACIFIC

Our work on the high-incidence foci of amyotrophic lateral sclerosis and Parkinson's disease has led to the further confirmation that in these places there is premature aging of the population with early appearance of neurofibrillary tangles in brain. We have now identified the pathogenic mechanism involved in these foci, which has been demonstrated at the epidemiological level to involve early life (in utero ontogenesis, infancy, childhood, adolescence) spent in environments enormously deficient in calcium and magnesium, in "primitive", isolated cultures with no outside food sources and from which the patients have never traveled. With the change in social and economic conditions after World War II in the Japanese Kii peninsula focus and among the Chamorro people on Guam, it is now clear that the calcium and magnesium deficiency no longer pertains and this accounts for the enormous decline in incidences of both diseases. No such decline has occurred in New Guinea, where the focus of both diseases is much more intense, except in one village; people in that village moved away from the region and changed their environmental exposure and economic status and were exposed to imported foodstuffs. This hypothesis is clearly substantiated by environmental analyses of soil, drinking water and foodstuffs.

IMAGING OF CALCIUM, ALUMINUM, AND SILICON AND OTHER CATIONS IN THE HUMAN BRAIN

Electron-probe X-ray microanalysis and neutron activation analysis have clearly demonstrated the deposition of calcium, aluminum and other di- and tri-valent cations in neurons, particularly those that develop neurofilbrillary tangles. Our recent (as yet unpublished) studies using the computer-controlled imaging X-ray microprobe have demonstrated the presence of silicon in the same NFT-bearing neurons containing calcium and aluminum. This is the first report of intraneuronal silicon deposition in Guamanian ALS and PD and extends the work of earlier investigators on the study of these elements in senile plaques and tangles in Alzheimer's disease. These techniques as well as infrared absorption and X-ray diffraction patterns suggest hydroxyapatite formation in affected neurons and now the posibility of aluminosilicate formation as well althought the exact specialtion of the mineral complexes have yet to be determined. We have now intensified our research efforts to include the elemental assessment of other neurofibrillary tangle, neurofilaments and amyloid plaque forming disorders including kuru, Creutzfeldt-Jakob disease, Gerstmann-Strassler syndrome, scrapie and Alzheimer's disease.

Thus, early parathyoid adjustment required for life in calcium-deficient environment renders the host vulnerable to heavy metal intoxication with deposition of heavy-metals and calcium in neurons and seems to lead to the premature aging of the brain (the appearance of neurofibrillary tangles), and degenerative disease syndromes of the CNS. The implications of these discoveries for the study of motor-neuron diseases, parkinsonism-dementia and of the aging process itself are enormous and have already influenced research.

IN SITU DNA HYBRIDIZATION STUDIES IN ALS

Our collaborative work on the use of viral nucleic-acid probes for demonstrating by in situ hybridization the presence of genomic copies of viruses in neurons has led to an extremely important discovery. By in situ hybridization, copies of viral genomes of poliovirus were identified in neurons of control subjects, rather than in Guamanian ALS and PD and American ALS brain specimens. This finding casts a shadow over that whole methodological approach to all virology of chronic human diseases.

SELF-LIMITED CYSTICERCOSIS EPILEPSY ON PRIMARY INVASION

Our studies on the introduction of cysticercosis into previously virgin populations of Papua New Guinea and West New Guinea demonstrated a self-limited form of grand mal epilepsy in older children and adults, which is undoubtedly caused by the larval migrans phase of pig tapeworm infestation at a period before real cysts have developed in the brain. This self-limited disease requires no antiepileptic therapy, and the patients are left with no further seizures and no other obvious sequelae. We are now following the situation to determine which patients will later develop calcified intracerebral cysts, breakdown of cysts, and intractable epilepsy or other brain syndromes requiring neurosurgical treatment or elaborate anticysticercus chemotherapy. We have developed a sensitive ELISA test, now in worldwide use, for studying cysticercosis in man and animals, and have recently improved this by the analysis of the antigens involved and the preparation of purer antigens. We have demonstrated in Southeast Asian epilepsy clinics, in areas like Bali where cysticercosis is highly prevalent, that this newly-appreciated diagnosis is probably the cause of much of the self-limited new epilepsy seen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 NS 01282-21 CNSS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neurobiology of Population Isolates: Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primitive Cultures

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D.C. Gajdusek, M.D.	Chief	LCNSS
Others:	Clarence J. Gibbs, Jr., Ph.D.	Deputy Chief	LCNSS
	David M. Asher, M.D.	Research Medical Officer	LCNSS
	Paul W. Brown, M.D.	Medical Director	LCNSS
	Ralph M. Garruto, Ph.D.	Senior Research Biologist	LCNSS

COOPERATING UNITS (if any)

AUSTRALIA: Dr. Timothy Asch, Australian National University, Canberra; Dr. Cyril Curtain, CSIRO, South Melbourne; Dr. Eric French, Mt. Eliza; Dr. Chev Kidson, Queensland Institute of Medical Research, Brisbane; (continued)

LAB/BRANCH

Laboratory of Central Nervous System Studies, Intramural Research Program

SECTION

INSTITUTE AND LOCATION

NINCDS, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
12	8	4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies of human biology of vanishing primitive societies focus on neurological development and learning patterns in diverse cultural experiments in the human condition found in such isolated groups. Opportunistic investigation of problems phrased by man in isolation is the basis of approach from which all our studies have evolved. Techniques of molecular biology, immunology, virology, endocrinology and biochemistry and field epidemiological, clinical, linguistic and behavorial studies in cultural isolates and genetic and/or geographically isolated primitive bands yield more easily interpretable data than in cosmopolitan societies. Data and specimens collected on expeditions to Micronesia, Polynesia, Solomon Islands, New Hebrides, New Guinea, Indonesia, South America, Asia and Africa are used. Studies on nutrition, reproduction, fertility, neuroendocrine influences on age of sexual maturation and aging, genetic polymorphisms, genetic distance, unusual and odd employment of the higher cerebral functions in language learning, cognitive styles, computation (calculation without words or numbers) and culturally modified sexual behavior elucidate alternative forms of neurologic functioning for man which we would be unable to investigate once the natural cultural experiments in primitive human isolates are amalgamated into the cosmopolitan community of man. Foci of high incidence of kuru, ALS/PD, epilepsy, spastic paraparesis, familial parkinsonism, other CNS degenerations, hysterical disorders, schizophrenia, neoplasms, goiter, cretinism, rheumatoid diseases, diabetes, asthma, chronic lung disease, malaria, filariasis, leprosy, cysticercosis, and other infections are investigated. Zoonoses such as hemorrhagic fever with renal syndrome in China, Japan, Korea, USSR, Scandinavia, and the Balkans are studied including these newly recognized Bunyamwera viruses in the U.S. Acquired immune deficiency syndrome studied by our group in 1950-1960 have been reinitiated. Human evolution and adaptability to high altitude, excessively wet or arid climes, variable food supply, mineral deficiencies, toxic exposures and responses to severe diseases or social/psychological stress are under investigation in appropriate population isolates.

COOPERATING UNITS: (continued)

OTHERS: Michael Alpers, M.D.; Sina Bahmanyar, M.D.; Mario Barragan, M.D.; Francois Cathala, M.D., Kwang-Ming Chen, M.D.; Chen-ting Chin, M.D.; Millicent Coker-Vann, Ph.D.; Judith Farquhar, M.A.; Peter Fetchko, M.A.; Father David Gallus; Dmitry Goldgaber; Steven Ono, M.S.; Robert G. Rohwer, Ph.D., Andres Salazar, M.D.;

AUSTRALIA: Dr. Robert L. Kirk, Australian National University, Canberra; Dr. Robert MacLennan, University of Sidney, Sidney; Dr. Colin Masters, University of Perth, Perth; Dr. Euan Scrimgeour, Doncaster; Dr. John Sheridan, Queensland Institute of Medical Research, Herston; Dr. Fiona Stanley, Perth Medical Center, Nedlands; Dr. Stephen Wurm, Australian National University, Canberra.

BOLIVIA: Dr. Mario Barragan, La Paz; Dr. Mario Michael Zamora, Department of Neurologia y Neurocirugia, La Paz.

BRAZIL: Prof. Helio L. de Oliveira, Universidade de Sao Paulo, San Paulo.

CANADA: Dr. Jack Hildes, University of Manitoba, Winnipeg; Dr. Otto Schaefer, National Health and Welfare, Edmonston.

COLOMBIA: Dr. R. Barrett, Cali; Dr. R. Bijo, Hospital San Andres, Tumaco; Dr. Alvaro Duenas, Department of Microbiology, University of Valle, Cali; Dr. H. Groote, National Institutes of Health, Bogota; Dr. G. San Martin, National Institutes of Health, Bogota; Dr. G. Toro, National Institutes of Health, Bogota; Dr. V. Zaninovic, Neurologic Clinic, University Hospital, Cali.

ENGLAND: Mrs. Elisabeth Beck, Institute of Psychiatry, London; Dr. M.C. Clarke, Agricultural Research Council, Compton; Prof. P.M. Daniel, Royal College of Surgeons, London; Dr. A.J. Duggan, Wellcome Museum of Medical Science, London; Dr. George Nurse, London.

FIJI: Mr. Ron Crocombe, University of South Pacific, Suva.

FINLAND: Dr. Juhani Lahdevirta, University of Finland, Helsinki.

FRANCE: Dr. Francoise Cathala, Hopital de la Saipetriere, Paris; Dr. Maurice Godelier, L'Ecole Pratique Des Hautes Etudes, Paris; Dr. Jean Guiart, Paris.

GERMANY: Dr. Freidrich Deinhardt, Max-van-Petteenkoffer Institute, Munich; Dr. Klaus Mannweiler, Henrich-Pette-Institut fur Virologie und Immunologie, Hamburg; Dr. Wulf Schiefenhovel, Max-Planck Institut fur Verhaltensphysiologie, Percha; Dr. Heinz Stephan, Max-Plank-Institut fur Hirnforschung, Frankfurt-am-Niederrad.

HUNGARY: Dr. Tibor Trencseni, Prof. of Internal Medicine, Orvosi Hetilap, Budapest.

INDONESIA: Dr. James D. Converse, NAMRU 2, Jakarta; Dr. Kenneth Dresser, Sengo, Irian Jaya; Father David Gallus, Misi Katolik, Jayapura; Dr. Surjadi Gunawan, Public Health Department, Jakarta; Dr. B.A. Kawengian, Dr. Soewahjudi, Public Health Department, Jakarta; Bishop Alphonse Sowada, Catholic Mission, Jayapura; Dr. Budi Subianto, Public Health Department, Jayapura; Dr. Julie Sulianti Saroso, Public Health Department, Jakarta; Father Frank Trenkenkshuh, Catholic Mission Asmat, Jayapura; Dr. Laode R. Tumade, Department of Public Health, Jayapura; Mr. Jeff Verstegen, Associated Mission Aviation, Jayapura.

ITALY: Marek and Allison Jablonko, Perugia.

KENYA: Dr. Leendert C. Vogel, University of Nairobi, Nairobi.

MEXICO: Dr. Julio Sotelo, Mexico City; Dr. Reinhart Ruge, Cuernavaca.

NETHERLANDS: Father Ben van Oers, Missiehuis, Tilburg; Dr. Jaap Goudsmit, University of Amsterdam, Dr. Fransje van der Waals, Amsterdam.

NEW HEBRIDES: Capt. John Barley, Treasury/Customs Department, Port Vila; Dr. Kirk Huffman, Cultural Centre, Port Vila; Dr. Rabi Ramdoyal, World Health Organization, Port Vila; Dr. Ratard, French Hospital, Port Vila.

NEW ZEALAND: Dr. R.W. Hornabrook, Wadestown, Dr. J.M. McKenzie, Univeristy of Otago, Dunedin; Dr. J.A.R. Miles, Otago; Dr. Alistair G.C. Renwick, University of Auckland, Auckland;

PAPUA NEW GUINEA: Dr. Michael Alpers, Institute of Medical Research, Goroka; Dr. H.A. Brown, Port Moresby; Rev. F. Fischer, Lutheran Mission, Okapa; Dr. J. Linsley Gressitt, Wau Ecology Institute, Wau; Richard Lloyd, Summer Institute of Linguistics, Aiyura; Mr. Ivan Mbagintao, J.K. McCarthy Museum, Goroka; Dr. Stuart Merriam, Highland Christian Mission, Yagusa; Dr. Jack Onno, Department of Public Health, Port Moresby; Dr. Kerry Pataki-Schweizer, University of Papua New Guinea, Port Moresby; Dr. Alan Tarutia, Public Health Headquarters, Konedobu; Dr. Jeffrey Tuvi, Boroko.

PEOPLES REPUBLIC OF CHINA: Dr. H.E. Ce, Hubei Province Medical School, Wuchang; Dr. C.C. Cnin, Hubei Province Medical School, Wuchang; Dr. Chin-min Hsiang, Virus Research Institute, Hupeh Medical College; Dr. H.E. Kwei, Hubei Province Medical School, Wuchang; Dr. Toa Hung, Department of of Electron Microscopy, Peking University, Peking; Dr. Zhi-Yi Xu, Department of Epidemiology, Shanghai 1st Medical College, Shanghai; Dr. Zheng, Virus Research Institute, Hubei Medical College, Hubei.

PERU: Dr. Carlos Monge, Universidad Cayetano Heredia, Lima; Dr. Tulio Velasquez, Instituto de Biologia de Andina, Lima.

PHILIPPINES: Dr. John Cross, NAMRU-2, Manila; Dr. Benjamin Catubay, Provincial Health Officer, Ilocos Norte; Dr. Martesio C. Perez, Universtiy of Philippines, Manila; Lt. Commander William Schroeder, NAMRU-2, Manila; Dr. Virginia Basaca Sevilla, Ministry of Health, Manila; Dr. Elizabeth Zaraspe-Yoo, University of Philippines, Manila.

MADAGASCAR: Dr. Pierri Coulanges, Institute Pasteur de Madagascar, Antanarivo.

MAURITIUS: Dr. B. Gurburrum, Ministry of Health, Port Louis.

REPUBLIC OF CHINA: Dr. R. Palmer Beasley, University of Washington, Taipei.

REUNION ISLAND: Dr. Charles Bosquet, Hopital de Terre Rouge, St. Pierre; Dr. Jean-Baptiste Dandelot, Hopital de Terre Rouge, St. Pierre; Dr. Maurice Jay, Hopital Psychiatrique, St. Paul.

SCOTLAND: Dr. Alan G. Dickinson, A.R.C. Animal Breeding Research Organization, Edinburgh; Dr. J.D. MacGregor, Shetland Health Board, Shetland.

SINGAPORE: Chong Keat Lim, Architects Team 3; Prof. Dr. Lim Kok Ann, Dr. Ivan Polunin, University of Singapore; Dr. Foo Keong Tatt, Singapore General Hospital.

SOLOMON ISLANDS: Dr. D. Mackay, Center Hospital, Honiara; Dr. A.M.O. Solomon, Health Department, Kirakira; Dr. B. Wilkin, Central Hospital, Honiara.

SWITZERLAND: Dr. Liana Bolis, World Health Organization, Geneva; Dr. Stephen Fazekas, Basel Institute for Immunology, Basel.

USSR: Dr. Mikhail Petrovich Chumakov, Institute for Poliomyelitis and Virus Encephalides, Moscow; Dr. Lydia L. Fadeeva, Ulitsa Valters Ulbrichta, Moscow; Dr. L.G. Goldfarb, Institute of Poliomyelitis and Viral Encephalitides, Moscow; Prof. Vera I. Il'yenko, All-Union Research Institute of Influenza, Leningrad; Prof. D.K. Lvov, D.I. Ivanovskii Institute of Virology, Moscow; Dr. Prokopii Andrevich Petrov, Iakut Ministry of Public Health, Iakutsk; Dr. Anatoli Alexandrovich Smordintsev, Leningrad; Dr. Victor Zhadanov, Ivanovskii Institute of Virology, Moscow.

UNITED STATES: Alabama--Dr. James Dutt, University of South Alabama, Mobile; Dr. Charles Hoff, University of South Alabama, Mobile; Dr. Wladimir Wertelecki, University of South Alabama, Mobile; Arizona--Dr. Tim Kuberski, National Institute of Arthritis, Metabolism, and Digestive Diseases, Phoenix; California--Mr. James Boykin, Valencia; Dr. L.L. Cavalli-Sforza, Stanford University, Palo Alto; Dr. David Lang, City of Hope Hospital, Duarte; Dr. Michael N. Oxman, V.A. Hospital, San Diego; Dr. Guy Pawson, University of California, San Francisco; Delaware--Dr. Roger Rodrique, Wilmington; Georgia--Dr. Carol Ballew, University of Georgia, Athens; Hawaii--Dr. Arwin Diwan, University of Hawaii, Honolulu; Dr. Leon Rosen, Pacific Research Center, Honolulu; Don Rubinstein, University of Hawaii, Honolulu; Illinois--Judith Farquhar, University of Chicago, Chicago; Maryland--Dr. Paul Hoffman, University of Maryland, Baltimore; Dr. Richard T. Johnson, Johns Hopkins Hospital, Baltimore; Dr. Guy McKhann, Johns Hopkins University, Baltimore; Dr. Chris Plato, Gerontology Research Center, Baltimore; Dr. Constantine Sakles, University Hospital, Baltimore; Dr. Charles Wisseman, University of Maryland, Baltimore; Dr. K.V. Shah, Johns Hopkins University, Baltimore; Massachusetts--Dr. John Enders, Brookline; Mr. Peter Fetchko, Peabody Museum, Salem; Michigan--Prof. J.V. Neel, University of Michigan, Ann Arbor; Dr. Ernst A. Rodin, Lafayette Clinic, Detroit; Minnesota-- Dr. Leonard Kurland, Mayo Clinic, Rochester; Dr. G. Albin Matson, Minneapolis;

Nevada--Dr. Warren V. Huber, V.A. Medical Center, Reno; New Jersey--Dr. Karl Maramorosch, Rutgers University, New Brunswick; Dr. Richard Masland, Englewood; New York--Dr. Robert Glasse, Queen's College, Flushing; Dr. Shirley Lindenbaum, The New School, New York; Dr. Ralph D. Peterson, New York Hospital-Cornell Medical Center, New York; Dr. Roger D. Traub, IBM Thomas W. Watson, Yorktown; Ohio--Dr. Richard Feinberg, Kent State University, Kent; Dr. Frank P. Saul, Medical College, Toledo; Dr. Arthur G. Steinberg, Case Western Reserve University; Pennsylvania--Dr. Paul T. Baker, Pennsylvania State University, University Park; Drs. Werner and Gertrude Henle, Children's Hospital of Philadelphia, Philadelphia; Rhode Island--Dr. Terrence E. Hays, Rhode Island College, Providence; Dr. John Strom, Rhode Island Hospital, Providence; Texas--Dr. Heather D. Mayor, Baylor University Medical School, Houston; Dr. Steven Wiesenfeld, Southwest Allergy Service, Inc., Midland; Washington--Dr. Ronald DiGiacomo, University of Washington, Seattle; Wisconsin--Dr. G.R. Hartsough, Great Lakes Mink Association, Pittsville; Dr. Richard F. Marsh, University of Wisconsin, Madison; Dr. Gabriel Zu Rhein, University of Wisconsin, Madison.

YUGOSLAVIA: Dr. A. Terzin, Department of Microbiology. Faculty of Medicine, Novisad; Prof. J. Vesenjak-Hirjan, Sveucilistau Zagrebu, Zagreb.

Sub-Project I:	Study of the development patterning of the human nervous system (cybernetics of human development).
Sub-Project II:	Human evolutionary studies in isolated primitive groups.
Sub-Project III:	Studies of isolated Micronesian populations.
Sub-Project IV:	Studies of isolated New Guinea populations.
Sub-Project V:	Studies of Australian Aborigines.
Sub-Project VI:	Studies of isolated New Hebrides and Solomon Islands populations.
Sub-Project VII:	Studies of Central and South American Indians.
Sub-Project VIII:	Developmental, genetic and disease patterns in primitive and isolated populations of Asia, Africa, Indonesia, Melanesia, Micronesia, Polynesia, South and Central America, and the Arctic.
Sub-Project IX:	Experimental developmental neuropediatrics in infantile programming: a empirical approach to the language of information input into the nervous system.
Sub-Project X:	Ciphers and notation for the coding of sensory motor data for neurological information processing.

Sub-Project XI:	Racial distribution and neuroanatomic variations in the structure of the human brain.
Sub-Project XII:	Studies of high incidence of neurological disease in specific racial and ethnic groups and in primitive, or geographically genetically, culturally, or socially isolated group population studies.
Sub-Project XIII:	Studies of high incidence of non-neurolgical disease in specific racial and ethnic groups and in primitive, or geographically genetically, culturally, or socially isolated group population studies.
Project Description:	Neurobiology of Population Isolates: Study of Child Growth and Development, Behavior and Learning, and Disease Patterns in Primitive Cultures (are attached)
Publications:	Listed on pages 32 - LCNSS/IRP through 37 - LCNSS/IRP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 00969-21 CNSS

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Chronic CNS Disease Studies: Slow, Latent and Temperate Virus Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D.C. Gajdusek, M.D.	Chief	LCNSS
Others:	Clarence J. Gibbs, Jr., Ph.D.	Deputy Chief	LCNSS
	David M. Asher, M.D.	Research Medical Officer	LCNSS
	Paul W. Brown, M.D.	Medical Director	LCNSS
	Ralph M. Garruto, Ph.D.	Senior Research Biologist	LCNSS

COOPERATING UNITS (if any) AUSTRALIA: Dr. Byron A. Kakulas, University of Western Australia, Nedlands; Dr. Chev Kidson, Queensland Institute of Medical Research, Brisbane; Dr. Robert L. Kirk, Australian National University, Canberra; Dr. Ian MacKay, Royal Melbourne Hospital, Melbourne; (continued)

LAB/BRANCH
Laboratory of Central Nervous System Studies, IRP, NINCDS

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
24	14	10

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies elucidate cause and pathogenesis of chronic degenerative CNS disorders with emphasis on MS, ALS, Parkinsonism-dementia, Parkinson's, Pick's, and Alzheimer's disease, Huntington's chorea, supranuclear palsy, other presenile dementias, spinocerebellar ataxias, epilepsy, chronic encephalitis with focal epilepsy, muscular dystrophies, chronic schizophrenia, autism, SSPE, PML, dialysis encephalopathy, and intracranial neoplasm. Even familial, apparently hereditary diseases may be slow virus infections. Subacute spongiform virus encephalopathies: kuru and Creutzfeldt-Jakob disease (CJD) of man; scrapie and mink encephalopathy are caused by unconventional viruses with unique properties posing important theoretical problems to microbiology and molecular biology; a major goal is elucidation of their structure and mechanisms of replication. Transmissible virus dementias are increasinly recognized worldwide causes of death: high incidence foci, transmission by corneal transplant or brain surgery, and occupational hazards from exposure to diseased or infectious brain. In order to determine the usual mode of infection with the virus, a worldwide epidemiological study of transmissible virus dementia (CJD) cases is underway with special attention to familial clusters of cases and with a quest for possible relationship of scrapie of sheep to the human disease.

Familial and nonfamilial dementia and the dementias of senility are studied. The autoimmune responses to specific brain antigens in CNS diseases are under intensive investigation. DNA in situ hybridization and electrophoretic focusing partition of proteins along with enzymatic and hybridoma immunofluorescence and many other techniques are used to try to identify viral subunits and partial genomes in tissues in chronic diseases.

PRINCIPAL INVESTIGATORS: (continued)

OTHER: Herbert L. Amyx, D.V.M.; David M. Asher, M.D.; Sina Baymanyar, M.D.; Paul W. Brown, M.D.; Chen-ting Chin, M.D.; Ryo Fukatsu, M.D.; Ralph M. Garruto, Ph.D.; Jaap Goudsmit, M.D.; D. Guiroy, M.D.; C.M. Hsiang, M.D.; Yasuo Kuroda, Ph.D.; Pyung-Woo Lee, Ph.D.; Maryellen F. Franko, Ph.D.; Carlo Masullo, M.D.; T. Nakamura, M.D.; C. Piccardo, M.D.; Maurizio Pocchiari, M.D.; Pamela Rodgers-Johnson, M.D.; Robert G. Rohwer, Ph.D.; Akira Takenaka, M.D.; Fransje van der Waals, M.D.; Richard T. Yanagihara, M.D.; Francoise Cathala, M.D., Dimitry Goldgaber, Ph.D.; Euan Scrimgeour, M.D.; Leon Epstein, M.D.

COOPERATING UNITS: (continued)

AUSTRALIA: Dr. Colin Masters, University of Western Australia, Perth; Dr. Eric Shaw, Red Cross Blood Transfusion Service, Brisbane; Dr. Margaret Sabine, University of Sydney, Sydney.

AUSTRIA: Prof. F. Seitelberger, University of Vienna, Vienna.

BELGIUM: Dr. A. Lowenthal, L'Institut Bunge, Antwerp.

CANADA: Dr. John H. Deck, Toronto Western Hospital, Toronto; Dr. Joseph Gilbert, University Hospital, London; Dr. Arthur J. Hudson, University Hospital; London; Dr. Andrew Kertez, St. Joseph's Hospital, London; Dr. Theodore Rasmussen; McGill University, Montreal; Dr. N.B. Rewcastle, Banting Institute, Toronto.

CHILE: Dr. Sergio Galvez, Institute de Neurocirurgia, Santiago.

REPUBLIC OF CHINA: Prof. Chi-lu Chen, National Taiwan University, Taipei; Dr. C.H. Yen, National Health Administration; Taipei; Dr. Chin-Yun Yii, Kaohsinung Medical College, Kaohsiung.

COLOMBIA: Dr. V. Zaninovic, Neurological Clinic, University Hospital, Cali; Dr. Alvaro Duenas, Department of Microbiology, University del Valle, Cali.

CUBA: Dr. Segundo Mesa-Castillo, Hospital Psiquiatrico de la Habana, Havana.

CZECHOSLOVAKIA: Dr. Vlastimil Mayer, Slovak Academy of Sciences, Bratislavia; Dr. Eva Mitrova, Research Institute for Preventative Medicine, Limbova.

EGYPT: Dr. Harry Hoogstraal, Naval Medical Research Unit, Cairo.

ENGLAND: Mrs. Elisabeth Beck, Institute of Psychiatry, London; Dr. M.C. Clark, Agricultural Research Council, Compton; Prof. P.M. Daniel, Royal College of Surgeons, London; Dr. A.J. Davey, Royal College of Medicine, Institute of Psychiatry, London; Prof. George Dick, Regional Dean's Office, London; Prof. L.W. Duchen, The National Hospital, London; Dr. D.A. Haig, Agriculture Research Council, Compton; Dr. Gordon D. Hunter, Agricultural Research Council, Compton; Prof. W.B. Matthews, University of Oxford, Oxford.

COOPERATING UNITS: (continued)

FINLAND: Prof. Nils Oker-Bloom, University of Helsinki, Helsinki.

FRANCE: Dr. Francoise Cathala, Hopital de la Salpetriere, Paris; Dr. Henri-Pierre Cathala, Hopital de la Salpetriere, Paris; Dr. Louis Court, Centre de Recherches du Service, Clamart; Dr. Michel Dumas, CHU, Limoges; Prof. A.E. Escourolle, Charles Foix La Salpetriere, Paris; Dr. Henri Gastaut, University de Marseille, Marseille; Dr. Patrick Gourmelon, CRSSA, Clamart; Dr. Raymond Latarjet, Institut du Radium; Paris; Dr. Martin, CHU, Nice; Dr. Francis Rohmer, CHU, Strasbourg; Dr. Michel Samson, CHU, Roen; Dr. Schott, CHU, Lyon; Prof. Tamalet, Hopital de La Timone, Marseille.

GERMANY: Dr. Conrad Beyreuter, Univ. of Cologne, Cologne; Dr. Freidrich Deinhardt, Max-van-Pettekoffer Institute, Munich; Dr. Heino Diringer, West Berlin; Dr. Klaus Mannweiler, Henrich-Pette-Institute fur Virologie und Immunologie, Hamburg; Dr. W.K. Muller, Country Psychiatric Hospital, Wiesloch; Dr. Volker ter Meulen, Institute fur Virologie, Wurzburg; Dr. Wolfgang Zeman, Lahstein.

GUAM: Dr. Kwang-Ming Chen, NINCDS Research Center, Tamuning; Dr. Leon Concepcion, Guam Memorial Hosptial, Agana; Dr. Olivia Cruz, NINCDS Research Center, Tamuning; Jose Torres, NINCDS Research Center, Tamuning.

ICELAND: Dr. Margret Gudnadottir, University of Iceland, Reykjavik; Dr. P.A. Palsson, University of Iceland, Reykjavik; Dr. G. Petursson, University of Iceland, Reykjavik.

ITALY: Prof. L. Amaduci, Dept. of Virolology, Firenze; Dr. C. Fieschi, Clinica Neurologica Universita, Rome; Dr. M. Nardini, Clinical Neurologica Universita, Rome; Prof. G. Macchi, Univ. Cattolica del S. Cuore, Rome; Dr. Carlos Masullo, Catholic University, School of Medicine, Rome; Dr. F. Orzi, Clinica Neurologica, Rome; Dr. Maurizio Pocchiari, Instituto de Clinica del Malatte Nervouse e Mentale, Faculta de Medicina e Chirurguria, Rome; Dr. F. Rocchi, Clinical Neurologica Universita, Siena.

INDONESIA: Dr. Budi Subianto, Public Health Department, Jayapura.

JAMAICA: Dr. D. Graham, Kingston Public Hospital, Kingston; Dr. J. Hall, Kingston Public Hopital, Kingston; Prof. Owen Morgan, Dept. of Med., Univ. of W. Indies, Kingston.

JAPAN: Dr. Tomonobu Aoki, Fukuoka; Dr. Fukatsu, Sapporo; Dr. Fushahiro Ikuta, Brain Research Institute, Niigata; Dr. Kiyotaro Kondo, Brain Research Institute, Niigata; Dr. Kuroda, Saga City; Dr. Yoshigoro Kuroiwa, Kyushu University, Fukuoka; Dr. Takao Makifuchi, Brain Research Institute, Niigata; Dr. Ryoichi Mori, Kyushu University, Fukuoka; Dr. Shigeru Mori, Brain Research Institute, Niigata; Dr. Nobuyuki Murakami, Nagoya University of School of Medicine, Nagoya; Dr. Seiho Nagafuchi, Kyushu University, Fukuoka; Dr. Nakamura, Nagasaki; Dr. Ikuya Nagata, Nagoya University, Nagoya; Dr. Hiroshi Oda, Kagoshima University, Kagoshima; Dr. M. Ohta, Kyushu University; Fukuoka; Dr. Takanaka, Kyushu Univ., Kyushu; Dr. S. Tanaka, Wakayama, Medical College, Wakayama; Dr. J. Tateishi,

COOPERATING UNITS: (continued)
JAPAN (continued):
Kyushu University, Fukuoka; Dr. Tadao Tsubaki, Tokyo Metropolitan Neurological Hospital, Tokyo; Dr. Y. Uebayashi, Wakayama Medical College, Wakayama; Dr. F. Yashimasu, Wakayama Medical College, Wakayama; Dr. M. Yasui, Wakayama Medical College; Wakayama; Dr. Yoshiro Yase, Wakayama Medical College, Wakayamashi.

KOREA: Dr. Ho Wang Lee, Korea University Medical College, Seoul; Dr. P.W. Lee, Korean University Medical College, Seoul.

MEXICO: Dr. Julio Sotelo, Institut Nacional de Neurologia, Mexico City.

NETHERLANDS: Dr. Jaap Goudsmit, University of Amsterdam, Amsterdam; Dr. Jan ten Brink, University Hospital of Amsterdam, Amsterdam; Dr. Jan van der Noordaa, Laboratorium voor Gezondheidsleer, Amsterdam.

NEW ZEALAND: Dr. R. W. Hornabrook, Wadestown.

PAPUA NEW GUINEA: Dr. Michael Alpers, Institute for Medical Research, Goroka.

PERU: Dr. Luis Palomino, Hospital Santo Toribio, Lima.

POLAND: Dr. P.P. Liberski, Department of Neurology, Lodz

PUERTO RICO: Dr. Victor Mojica, Veterans Administration Center, San Juan.

PEOPLES REPUBLIC OF CHINA: Dr. Chen-ting Chin, Beijing; Dr. Chin-Yun Yii, Kaohsinung Medical College, Kaohsiung.

SCOTLAND: Dr. Alan G. Dickinson, A.R.C. Animal Breeding Research Organization, Edinburgh; Dr. Hugh Fraser, A.R.C. Animal Breeding Research Organization, Edinburgh; Dr. Richard Kimberlin, Univ. of Edinbugh, Edinburgh; Dr. J.D. MacGregor, Shetland Health Board, Shetland.

SOUTH AFRICA: Dr. J.H.S. Gear, National Institute of Virology, Sandringham.

SPAIN: Dr. J.A. Sanchez-Martin, Instituto de Investigaciones, Madrid; Dr. Alberto Portera-Sanchez, Cuidad Savitaria Primero, Madrid.

SWEDEN: Dr. Erling Norrby, Karolinska Institute, Stockholm; Dr. Arne Svedmyr, Central Bacteriological Laboratory, Stockholm.

SWITZERLAND: Dr. Christoph Bernoulli, Universitspital, Zurich; Dr. Liana Bolis, World Health Organization, Geneva; Dr. Breget, University of Geneva, Geneva; Dr. B. Ney, University of Geneva, Geneva.

USSR: Dr. Mikhasil Petrovich Chumakov, Institute of Poliomyelitis and Virus Encephalides, Moscow; Dr. Lydia L. Fadeeva, Ulitsa Valtera Ulbrichta, Moscow; Dr. Sophia Janovna Gaidamovich, Ivanovskii Institute of Virology, Moscow; Prof. Vera I. Il'yenko, All-union Research Institute of Influenza, Leningrad; Dr. Prokopii Andrevich Petrov, Iakut Ministry of Public Health, Iakutsk; Dr. Vanda V. Pogodina, The Institute of Poliomyelitis and Virus Encephalitides, Moscow; Dr. Peter Rytik, Bilorussioan Institute of Epidemiology, Minsk.

COOPERATING UNITS: (continued)

UNITED STATES: California--Dr. David E. Kohne, Center for Neurologic Studies, San Diego; Dr. Peter Lampert, University of California, La Jolla; Dr. David Lang, City of Hope Medical Center, Duarte; Dr. Michael N. Oxman, V.A. Hospital, San Diego; Dr. Linus Pauling, Linus Pauling Institute, La Jolla; Dr. Stanley Prusiner, University of California, San Francisco; Dr. Gunther Stent, University of California, Berkeley; Dr. Robert Terry; San Diego; Dr. W. W. Tourtellotte, V.A. Hospital, Los Angeles; Dr. Myron Varon, Amyotrophic Lateral Sclerosis Society, Sherman Oaks; Dr. Steven Waxman, Stanford University, Stanford; Dr. Leslie P. Weiner, University of Southern California, Los Angeles. Connecticut--Dr. P. N. Bhatt, Yale University, New Haven; Dr. G.D. Hsiung, V.A. Medical Center, West Haven; Dr. Elias and Laura Manuelides, Yale University School of Medicine; New Haven. Hawaii--Dr. Arwin R. Diwan, University of Hawaii, Honolulu; Dr. Hong-Yi Yang, University of Hawaii, Honolulu. Illinois--Dr. Raymond A. Classen, Presbyterian-St. Lukes's Hospital, Chicago; Dr. Raymond Roos, University of Chicago, Chicago. Indiana--Dr. Bernadino Ghetti, Indiana University School of Medicine, Indianapolis; Dr. Morris Pollard, Lobund Laboratory, Notre Dame; Dr. A.N. Siakotos, Indiana University, Indianapolis. Kentucky--Dr. Dan Tynan, V.A. Hospital, Lexington. Louisiana--Dr. William Greer, Gulf South Research Institute, New Iberia. Maryland--Dr. Dan C. Cavanaugh, Rockville; Dr. Theodore O. Diener, Agricultural Research Center West, Beltsville; Dr. Paul Hoffman, University of Maryland; Baltimore; Dr. Kenneth P. Johnson, Dept. of Neurol., University of Maryland, Baltimore; Dr. Richard T. Johnson, Johns Hopkins University, Baltimore; Mrs. Meta Neumann, Bethesda: Dr. Robert Traub, University of Maryland, Baltimore; Dr. Charles Wisseman, University of Maryland, Baltimore; Dr. K.V. Shah, Johns Hopkins University, Baltimore; Mr. T.C. Rains, National Bureau of Standards, Gaithersburg. Massachusetts--Dr. Amico Bignami, Children's Hospital Medical Center, Boston; Dr. Bernard Fields, Harvard Medical School, Boston; Dr. E. P. Richardson, Jr., Massachusetts General Hospital, Boston; Dr. W.C. Schoene, Peter Bent Brigham Hospital, Boston. Minnesota--Dr. Ashley T. Haase, University of Minnesota, Minneapolis; Nevada--Dr. Warren V. Huber, V.A. Medical Center, Reno. New York--Dr. Samuel J. Ayl, The National Foundation March of Dimes, White Plains; Dr. Jordi Casals, Mt. Sinai School of Medicine, New York; Dr. Teresita S. Elizan, Mt. Sinai School of Medicine, New York; Dr. Scott Halstead, Rockefellar Foundation, New York; Dr. Asao Hirano, Montefiore Hospital, Bronx; Dr. John Hotchin, Department of Health, Albany; Dr. Imaharu Nakano, Montifiore Hospital and Medical Center, New York; Dr. Michael L. Shelanski, New York University Medical Center, New York; Dr. Roger D. Traub, IBM Thomas B. Watson Research Center, Yorktown Heights; Dr. James D. Watson, Cold Spring Harbor Laboratory, Cold Spring. Ohio--Dr. S.M. Chou, Cleveland Foundation, Cleveland; Dr. Maurice Victor, Metropolitan General Hospital, Cleveland. Texas--Dr. Samuel Baron, University of Texas, Galveston; Dr. Steven Wiesenfeld, Southwest Allergy Service, Midland. Virginia--Dr. J. L. Hourrigan, Arlington. Washington--Dr. Ellsworth C. Alvord, Jr., University of Washington, Seattle. Washington, D.C.--Dr. Harold Booker, Veterans Administration Central Office, Washington; Dr. John Kurtzke, V.A. Hospital, Washington; Dr. Frederick C. Robbins, National Academy of Science, Washington;

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UNITED STATES: (continued)
Washington, D.C. (continued): Dr. Fuller Torrey, St. Elizabeth's Hospital, Washington. Wisconsin--Dr. Richard F. Marsh, University of Wisconsin, Madison; Dr. Gabriel Zu Rhein, University of Wisconsin, Madison.

YUGOSLAVIA: Dr. A. Gligic, Institute of Immunology and Virology, Beograd; Dr. Miha Likar, Mikrobioloski Institut, Ljubljana; Dr. D. Terzin, Institute of Virology, Serajevo; Prof. J. Vesenjak-Hirjan, University of Zagreb, Zagreb.

Sub-Project I: Attempts to isolate, identify and characterize transmissible agents from humans and animals with subacute degenerative diseases of the central nervous system: transmissible heredofamilial diseases, presenile and senile dementias of the sporadic and familial types and primary sclerosing and demyelinating diseases.

Sub-Project II: Characterization and pathogenesis of kuru virus.

Sub-Project III: Characterization and pathogenesis of Creutzfeldt-Jakob disease (transmissible dementia virus).

Sub-Project IV: Scrapie: studies on the purification, physical and biological characterization and nature of the virus.

Sub-Project V: *In vitro* cultivation of the viruses of the subacute spongiform virus encephalopathies in cell cultures.

Sub-Project VI: Host range of susceptible laboratory animals to the viruses of the subacute spongiform virus encephalopathies.

Sub-Project VII: Strain variations among the viruses of the subacute spongiform virus encephalopathies.

Sub-Project VIII: Cell-fusing properties of the viruses of the subacute spongiform virus encephalopathies.

Sub-Project IX: Resistance to radiation of the viruses of the subacute spongiform virus encephlopachies.

Sub-Project X: Resistance to disinfectants of the viruses of the subacute spongiform virus encephalopathies.

Sub-Project XI: Tissue and cell culture techniques used to unmask slow infection of man and animals using brain and viscera biopsy and early autopsy, bone marrow and peripheral leucocyte specimens.

Sub-Project XII: The syncytium-forming viruses (simian and human foamy viruses).

Sub-Project XIII: Studies on transformed human brain tissue *in vitro* and characterization of associated virus.

Sub-Project XIV: Electron microscopic membrane studies of subactue spongiform virus encephalopathies.

Sub-Project XV: Characterization and identification of new herpes viruses from explant cultures of tissues from subhuman primates.

Sub-Project XVI: Studies on persistent asymptomatic cytomegalovirus infections of healthy rhesus monkeys.

Sub-Project XVII: Focal movement disorders in rhesus monkeys following experimental infection with a strain of tick-borne encephalitis virus.

Sub-Project XVIII: Fluorescent antibody studies on the intracellular localization and identification of virus antigens *in vivo* and *in vitro* in tissues from patients with subacute diseases of the central nervous system.

Sub-Project XIX: Isolation and characterization of adenovirus from the urine of chimpanzees.

Sub-Project XX: Development of serological and immunological test system for use in the study of slow infections of the central nervous system.

Sub-Project XXI: Immune responsiveness of multiple sclerosis patients to established viral antigens by detection of specific antibodies in serum and cerebrospinal fluids collected serially during remission and exacerbation.

Sub-Project XXII: Animal management and intercurrent diseases in subhuman primates on long-term studies of slow infections.

Sub-Project XXIII: Studies to determine the possible presence of cryptic viral genomes in human brain tissues.

Sub-Project XXIV: Sequential development of kuru-induced neuropathological lesions in spider monkeys.

Sub-Project XXV: Studies on the isolation, characterization, identification and pathogenicity of type C viruses from human and animal tissues.

Sub-Project XXVI: Biochemical studies of the etiology of amyotrophic lateral sclerosis and parkinsonism-dementia.

Sub-Project XXVII: Study of mitrochrondrial mutants from scrapie-infected mouse brain cells.

Sub-Project XXVII: Study of mitrochrondrial mutants from scrapie-infected mouse brain cells.

Sub-Project XXVIII: Isolation and characterization of the etiological agent of Scandinavian nephro-nephritis epidemica.

Sub-Project XXIX: The pathogenesis of Korean hemorrhagic fever virus and the elucidation of its biological and physical properties.

Sub-Project XXX: Worldwide seroepidemiological evidence of antibodies in human populations to the virus of Korean hemorrhagic fever.

Sub-Project XXXI: Development of an enzyme-linked immunoadsorbent (ELISA) test for the diagnosis and epidemiology of cystercercosis-induced epilepsy.

Sub-Project XXXII: Studies on the cytochemical and morphological properties of neurons cultured in vitro.

Sub-Project XXXIII: Development of immunological markers for the detection of autoantibodies to neurofilaments in the sera of patients with subacute spongiform encephalopathies.

Sub-Project XXXIV: Studies to determine the neurophysiological changes of neurons in vitro infected with CJD.

Sub-Project XXXV: Effects of the subacute spoingiform viruses on nerve cells grown in vitro.

Sub-Project XXXVI: In vivo and in vitro studies to determine the etiology of myasthenia gravis, Viliuisk encephalomyelitis and ALS-PD in high incidence foci of the Western Pacific.

Sub-Project XXXVII: Neurophysiological study of animals experimentally infected with subacute spongiform virus encephalopathies.

Sub-Project XXXVIII: Studies on in vivo pathogenecity of the retroviruses related to AIDS: HTLV (Gallo); French LAV-LOISEAU virus (Montagnier)

Sub-Project XXXIX: Attempts to transmit or isolate in vitro an etiological agent from AIDS, from pre-AIDS patients with lymphadenopathy syndrome, and from encephalitis associated with AIDS.

Sub-Project XXXX: Isolation and characterization of "unconventional viruses" (CJD) from multiple lots of human pituitary growth hormone.

Sub-Project XXXXI: Epidemiology of progressive degenerative disease of the CNS in receipients of human pituitary growth hormone.

Sub-Project XXXXII:	Development of procedures to exclude "unconventional viruses" from preparations of human pituitary growth hormone.
Sub-Project XXXXIII:	Preparation and characterization of synthetic polypeptides for scrapie, kuru, CJD and core protein of amyloid plaques in Alzheimer's disease.
Sub-Project XXXXIV:	Studies on the effects of altered slow axonal floow in the pathogenesis of subacute progressive degenerative disease of the nervous system.
Sub-Project XXXXV:	Studies on the deposition and distribution of heavy metals and essential minerals in central nervous system tissue from patients with progressive neurodegenerative disorders.

Project Description: Chronic Central Nervous System Disease Studies (described fully on pages 1-LCNSS/IRP through 16-LCNSS/IRP).

The projects (I through XXXXV) listed herein, as itemized in the Project Reports of previous years, have continued throughout this year and have been expanded, as are reflected in the extensive list of publications. Contractural phases of this work are being conducted at Gulf South Research Institute, New Iberia, LA.

Publications: Pages 32-LCNSS/IRP through 37-LCNSS/IRP

Publications In Print:

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CONTRACTS

University of Southwestern Louisiana
New Iberia Research Center
New Iberia, Louisiana

Contract #N01-NS-8-00931

$$91,660.00

Program Resources, Inc.
(Administration by NCI)

Contract #N01-CO-75380

$420,000.00.

TAB 4 -- LABORATORY OF EXPERIMENTAL NEUROPATHOLOGY -- (LENP)

ANNUAL REPORT

October 1, 1984 through September 30, 1985

Laboratory of Experimental Neuropathology

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ANNUAL REPORT
October 1, 1984 through September 30, 1985
Laboratory of Experimental Neuropathology, IRP
National Institute of Neurological and Communicative
Disorders and Stroke

Henry deF. Webster, Chief

The Laboratory of Experimental Neuropathology (LENP) includes the Cellular Neuropathology Section (CN) and the Neurotoxicology Section (NT). The main goal of the Laboratory's research program is to investigate cellular mechanisms of myelin breakdown, especially those that are directly related to multiple sclerosis and other human demyelinating diseases. During the past year, important discoveries have been made by scientists in both sections.

Cellular Neuropathology Section

1. Herpes Simplex Virus Type 2 (HSV-2) Pathogenesis and CNS Demyelination

This project has three general aims: (i) To define selected aspects of the pathogenesis of HSV-2 infections in mice, with particular attention to effects in the CNS, (ii) To further define conditions under which CNS demyelination occurs, and (iii) To further refine and test a working hypothesis which relates HSV-2 infection to the CNS demyelinative disease, multiple sclerosis (MS).

Published background studies from this laboratory include:(i) The first evidence that HSV-2 can produce an acute multifocal CNS demyelinative disease in mice which mimics certain pathological features of MS. The HSV-2 strain used in these experiments was originally isolated from the brain of a patient dying of typical chronic relapsing MS. (ii) Evidence that HSV-2 can produce non-fatal CNS demyelination by a natural genital route of infection. (iii) Evidence which suggests that tract-associated demyelinative lesions could arise by virus spread from a minimal neuronal infection via axonal transport and amplification of infection in the white matter. (iv) An examination of the epidemiologies of MS and HSV infections which suggest that MS could be a low-frequency complication of HSV-2 infection in persons lacking previous protective HSV-1 immunity. These studies are important because they address with some success several of the major plausibility issues which any specific etiological hypothesis for MS must satisfy.

Experiments undertaken, in progress, or completed in FY 1985 address important unresolved questions, including (i) whether and under what experimental conditions HSV-2 reactivates in the CNS, and (ii) whether demyelination is the consequence of CNS reactivation. Preliminary data raised the possibility that a virus-induced immunosuppression might be present at the severe end of the spectrum of HSV-2 infecton, and if that is so, this might be the context in which recurrent CNS demyelination might develop. Preliminary data which raised this possibility included virus presence in, and histological alterations of, lymphoid tissues. These findings appeared to be restricted to animals with severe infections and neurological disease.

Studies to further define the HSV-lymphoid tissue interaction and its relation to CNS disease include: (i) a survey of lymphoid tissues of adult mice at selected ages for virus presence by virus isolation, antigen methods, and ultrastructure. This study shows virus presence by isolation methods. The minimal amounts of virus present are consistent with either restricted virus replication in lymphoid tissues or virus circulation to lymphoid tissues from peripheral sites of replication. Further studies are needed to clarify this point. (ii) Completion of a study of sites of virus presence and replication in very young mice. In these animals, it is clear that virus replicates in lymphocytes, macrophages, and a variety of other lymphoid cells.

To test the hypothesis that immunosuppression may set the stage for recurrent CNS demyelination, we have begun a series of immunosuppression experiments on mice which have survived acute stage infections with CNS demyelination. Preliminary results show that with immunosuppression, virus can again be recovered from the genitourinary tract and the CNS. We will examine the CNS of such mice for presence of viral antigen and associated lesions.

We are also involved in two studies with clear and direct relevance to MS. (i) With Thomas Flynn and Dr. W.R. Green, Johns Hopkins, we are examining the eyes and optic nerves obtained at autopsy from patients dying of MS. This study defines a variety of retinal lesions and abnormalities which have never been well defined pathologically, and may provide useful insights into the pathogenesis of this aspect of MS. (ii) With Dr. Thomas Feasby, London, Ontario, we will examine serial sera from patients whose initial sign of disease is optic neuritis. Sera will be screened for seroconversion and rising viral antibodies. Sera are being collected.

2. Myelin Basic Protein and Multiple Sclerosis

There are two plausible theories concerning the etiology of multiple sclerosis (MS). The first postulates the persistence of a viral genome in the central nervous system. The occasional reactivation of the virus could either cause a direct cytopathic effect or could lead to immunopathological tissue damage. The second theory postulates the induction of autoimmunity to a myelin component by an antecedent viral infection. The latter infection need not involve the CNS. At present there is no hard evidence for either mechanism. With respect to the first, no viral genome or viral antigens have been consistently associated with the CNS of MS patients. With respect to the second, neither the immunological "trigger" nor the target antigen in the CNS have been identified. In this frustrating situation any information suggesting new lines of inquiry is valuable and should be investigated. A new lead has developed from recent studies in our laboratory on the structure of MBP, the major myelin protein which induces the experimental demyelinating disease, allergic encephalomyelitis (EAE). These findings implicate for the first time the papova virus T-antigens as candidates for a role in demyelinating disease. T-antigens are early, DNA-binding, regulatory proteins found primarily in the nucleus of papova virus infected cells. The T-antigens of JC and BK viruses share a threonine (Thr) phosphorylation site with MBP (Pro Arg/Lys Thr(P) Pro Pro Pro). Since JC virus productively infects the CNS in the demyelinating disease progressive multifocal leukoencephalopathy (PML), a latent or abortive infection of oligodendrocytes by JC with expression limited to the early T antigen could provide a mechanism for demyelination in which T-antigen competes for the MBP protein kinase. This would be a "theory 1" mechanism. On the other hand, serological studies show that both JC and BK could induce immunity to the Pro Lys Thr Pro Pro Pro sequence which might cross-react with the Pro Arg Thr Pro Pro Pro sequence of MBP. To pursue these questions we first developed a sensitive immunocytochemical method for the detection of T-antigen in frozen sections of PML CNS, and have also applied it to nine cases of MS. T-antigen was readily detected in five PML brains, but was not found in the MS tissue. Therefore, the mechanism of "theory 1" has been eliminated for papova viruses. However, in PML tissue T-antigen is present in many more cells than express virion antigens. This tends to strengthen the probability of an immune response to T-antigen during primary infections, or during JC or BK reactivations in the kidney. Thus, a "theory 2" mechanism must be considered for JC and BK viruses. Such a mechanism is currently under investigation.

3. Immunocytochemical Studies of HSV-2 Distribution and Myelin Protein Expression

This project has included further tests of postembedding immunocytochemical methods for light and electron microscopic localization of viral antigens and myelin proteins. These tests have characterized the effects of epon section pretreatments of tissue fine structure and on distribution of HSV-2 immunoreactivity in sections of cultured cells infected with this virus. In hydrogen peroxide-pretreated semithin and thin sections, cellular and organelle structures are well preserved and reaction product is localized on HSV-2 virions after the use of the PAP immunostaining procedure. On the other hand, use of sodium ethoxide in concentrations that removed significant amounts of epon during pretreatment also produced severe changes in cell and organelle structure. In addition, anti-HSV-2 immunoreactivity was not consistently observed on virions. Our conclusion is that localizations achieved with the use of sodium ethoxide (such as those described in some light microscopic studies of myelin-associated glycoprotein) may not be valid because sodium ethoxide can change tissue structure and can alter immunoreactivity distribution. Preliminary experiments have also tested the usefulness of Lowicryl as an embedding medium for the light and electron microscopic immunocytochemical localization of the P_o myelin glycoprotein in developing PNS tissue. Preliminary results show that it can be demonstrated in aldehyde-fixed PNS without section pretreatment. If confirmed and extended to thin sections examined electron microscopically, this embedment will be tested extensively for use in correlative immunocytochemical and nucleic acid hybridization studies.

Another important study in this project has been a quantitative immunocytochemical comparison of P_2 and P_1 myelin basic protein expression in developing rat VI nerves using specific antisera and the PAP method on epon-embedded sections. Anti-P_1 and anti-P_2 immunoreactivities were first detected on day 2 and the percentage of myelinated fibers immunostained rose rapidly, $P_1 \gg P_2$. By day 4, anti P_1 immunoreactivity was present in 95% of myelinated fibers; only 35% were stained by anti-P_2. For P_2, staining intensity and the percentage of immunostained sheaths continued to increase. Staining intensities were not uniform in identically processed sections. Intensity variation was greater with P_2 than with P_1. Quantitative results suggest that the intensity of anti-P_2 immunoreactivity correlates better with the amount of myelin present than with axon diameter. Differences in detection of immunoreactivity and its intensity probably reflect relative amounts of this minor PNS myelin constituent that can be detected by the method rather than selective expression of P_2 by Schwann cells myelinating large axons as previously reported. These results are important new findings because P_2 protein is thought by some to have a role in the immunologically mediated demyelination associated with the human disease, idiopathic polyneuritis.

Neurotoxicology Section

1. In Vitro Studies of Erythrosin B Neurotoxicity

Clinical, anecdotal, and experimental claims suggest that food dyes may have neurobehavioral effects on some children and young animals. We have previously reported that erythrosin B (tetraiodofluorescein, U.S.F.D. and D. Red No. 3), a commonly-used artificial food and drug color: 1) blocks synaptosomal uptake of dopamine; 2) inhibits ATP catalysis by brain and lamb kidney Na, K-ATPase and also sodium independent ATPases in brain membrane preparations; 3) inhibits the binding of the cardiac glycoside, ouabain, to brain Na,K-ATPase; 4) interacts with rat brain cortical membranes in a "receptor-like" manner; and 5) we have demonstrated a light-enhanced increase in the dye's potency to inhibit both [^{3}H]ouabain binding to Na,K-ATPase and ATP catalysis in rat brain membrane preparations.

Last year we hypothesized that erythrosin B is an in vitro toxic substance for a variety of physiological processes and is presumably a modulator of membrane structure, in general, and not (as previously believed) a specific inhibitor of that Na,K-ATPase exclusive to brain. In our most recent studies we have found that in bright light erythrosin B inhibits not only [^{3}H]ouabain binding to rat brain Na,K-ATPase but also both [^{3}H]cyclohexyladenosine binding to rat brain cortical membranes. However, we have seen no inhibition of [^{3}H]spiperone or [^{3}H]cyclohexyladenosine binding by erythrosin B in light-protected samples. These data suggest that the light-enhanced inhibitory actions of erythrosin B may be neurotoxic for a variety of membrane functions but the dark phase inhibition may be specific to ATPases. The manner in which it interacts with membranes remains to be clarified.

2. Anticonvulsant drugs, seizure disorders, and specific adenosine receptors

Interactions with CNS benzodiazepine receptors appear to be the molecular mechanism of actions for barbiturates and anticonvulsants of the benzodiazepine class. On the other hand, for many other anticonvulsant drugs the molecular mechanism of action has not been defined. Since adenosine and adenosine analogs have anticonvulsant effects in rat and mouse, we have been investigating the possibility that some clinically used anticonvulsants exert their effect by binding to central adenosine receptors.

We have used in vitro assays to measure the characteristics of CNS adenosine receptors in rat and guinea pig brain. The present study confirms and extends earlier data on carbamazepine interactions at adenosine receptors. The anticonvulsant is much more potent at the inhibitory A_1 adenosine receptor than at the stimulatory A_2 adenosine receptor. Despite extensive studies comparing and contrasting the effects of anticonvulsants, convulsants, and adenosine analogs on [^{3}H]CHA binding to the high affinity vs. the low affinity A_1 adenosine receptors, we have been unable to classify carbamazepine as either a pure agonist or pure antagonist at A_1 adenosine receptors. In addition, GTP appears to decrease the total number of available A_1 adenosine receptors rather than to cause a general shift of all receptors to a low affinity state.

Carbamazepine is clearly an antagonist at the A_2 receptor. In view of its structure we would expect it to be an antagonist at A_1 receptors as well. However, agonist activity at adenosine receptors would be more compatible with the sedative and anticonvulsant effects of carbamazepine, since potent A_1 adenosine agonists are sedative, while adenosine receptor antagonists, in particular, the methylxanthines are central stimulants. The present data suggest that interaction of carbamazepine with central A_1 adenosine receptors occurs at therapeutic doses, while equivalent interactions at A_2 receptors would require fourfold higher concentrations. The relationships between adenosine receptors and the anticonvulsant activity of this class of compounds require further investigation. Our studies will promote a better understanding of the convulsant and anticonvulsant properties of drugs, which clarify the directions for further biomedical research and lead to therapeutic improvements.

3. Neurotoxicity Mechanisms Studied in a Chromaffin Cell System

The chromaffin cell provides a well-characterized system for investigating molecular and cell-surface mediated mechanisms of neurotoxin action. Since several neurotoxins of interest to neurology are divalent cations (lead, manganese, copper, etc.) and since the storage vesicles of these cells, the chromaffin granules, contain high concentrations of calcium, these preparations have been investigated to determine the effect of toxic cations on calcium-mediated storage and release processes. Release of neurotransmitters and neuromodulators from their storage organelles takes place by exocytosis, a process in which the influx of calcium into the cell or nerve terminal triggers the fusion of the storage granule with the cell plasma membrane. The membrane fusion events can be modelled by studying the calcium-promoted fusion of artificial or biological membranes with each other.

The storage vesicles of chromaffin cells, chromaffin granules, accumulate large concentrations of catecholamines and ATP via carriers linked to the granule membrane Ca^{2+}-ATPase. Granule membranes contain an F_1 ATPase subunit which is highly similar to that of mitochondria. The catecholamine carrier is inhibited by reserpine while the ATP carrier is inhibited by atractiliside. The tricyclic antidepressants imipramine and chlorimipramine were examined for their effect on ATPase activity. While both drugs inhibited the activity of whole mitochondria, sub-mitochondrial particles and solubulized F_1-ATPase, they had little effect on whole granule or granule ghost enzyme activity.

Chromaffin granules will aggregate and fuse in the presence of calcium. We have been exploring the molecular basis of these activities. Labelling studies indicate that granule-granule recognition and aggregation is mediated by intrinsic membrane proteins. Fluorescent-labelled lipid probes have been successfully inserted into chromaffin granule membranes in vitro without altering the storage properties of the particles. Resonance energy transfer studies of calcium-promoted fusion of these membranes show that, unlike artificial phospholipid vesicles, fusion runs 5-10 fold slower than aggregation. These results imply that substantial rearrangement of the proteins and lipids of the membrane is required for fusion to occur. Fusion ability is lost if the granules are lysed and resealed; although other functions remain, e.g., uptake of catecholamines and ATP, maintainance of ion and pH gradients and ATP use activity. These results also suggest interactions of specific proteins are required for fusion to occur.

A multichannel, computer controlled stopped-flow rapid mixing spectrometer has been constructed to study these reactions and tested on a variety of artificial and biological membranes. We have developed a new assay for membrane fusion, based on monomer/exomer formation between chain-labelled pyrene phosphatidylcholine, which solves many problems encountered with energy transfer assays.

Various proteins and polypeptides can catalyse fusion of artificial vesicle membranes. Some of these proteins have known functions in biological systems (e.g., the spike protein from Semliki Forrest virus). SFV is closely related to rabies virus; therefore in vitro studies of protein-catalyzed membrane fusion mechanisms may have clinical relevance. The model polypeptide polylysine will fuse small unilamellar vesicles under conditions similar to SVF spike protein-mediated virus/cell membrane fusion. Recent stopped-flow studies indicated that polylysine-mediated fusion is aggregation rate limited. Furthermore, the aggregation rates themselves approach the diffusion-controlled limit. This implies that polylysine binds rapidly to the membrane-controlled limit. This implies that polylysine binds rapidly to the membrane surface(s) and that almost every collision of activated particles results in fusion. Myelin basic protein (MBP) also is a membrane-fusion catalyst. Preliminary experiments with MBP show aggregation rate-limited fusion of phospholipid vesicles at neutral pH. Similar experiments using SFV spike protein as catalyst are planned.

4. Myelin Basic Protein Conformation Studies

Myelin basic protein has been considered to exist in solution as a random coil with no long range order. Our recent experiments show that the naturally fluorescent amino acids of MBP exist in ordered structure. The protein binds ANS derivatives with Kd's of 10^{-6} tp 10^{-5} M. Porphyrins also bind with Kd's of $- 10^{-6}$ M and competitively displace ANS. These results argue for a high degree of 3-dimensional structural specificity of MBP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01995-13
LENP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Morphological Studies of Myelin Formation, Breakdown and Regeneration

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H. deF. Webster	Chief	LENP	NINCDS
Others:	A.F. Hahn	Visiting Scientist	LENP	NINCDS
	J.T. Favilla	Biologist	LENP	NINCDS

COOPERATING UNITS (if any)

Department of Biochemistry, McGill University, Montreal, Canada, (Drs. P. Braun, D. Frail), Department of Neurology, U. of Tenn., Medical School, Memphis, TN, (Dr. John Whitaker)

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Section on Cellular Neuropathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	1.5	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The long range goal of this project is to combine immunocytochemical methods with light and electron microscopy to study cellular mechanisms on myelin formation, breakdown, and regeneration. Current studies and major findings are : (1) Tests of postembedding light and electron microscopic immunocytochemical methods to evaluate: (a) effects of epon section pretreatments on tissue fine structure and on distributions of herpes virus type 2 (HSV-2) immunoreactivity in sections of cultured cells infected with this virus. In H_2O_2 pretreated semithin and thin sections, cellular structure is well preserved and reaction product is located on HSV-2 virions after use of either the peroxidase-anti-peroxidase (PAP) or the biotin-avidin immunostaining procedure. Use of sodium ethoxide in concentrations that removed significant amounts of epon during pretreatment also severely altered cell and organelle fine structure; therefore, this pretreatment agent could not be used to study distribution of immunoreativity electron microscopically. (b) Usefulness of Lowicryl as an embedding medium for light and electron microscopic localization of the P_0 myelin glycoprotein in developing PNS tissue. Preliminary results indicated that it can be demonstrated in aldehyde-fixed PNS without section pretreatment. If confirmed and extended to thin sections examined electron microscopically, this embedment will be tested extensively for use in nucleic acid hydridization studies. (2) Immunocytochemical comparison of P_2 and P_1 basic protein expression in Schwann cells of developing rat VI nerves. Immunoreactivity was first detected on day 2 and the percentage of myelinated fibers immunostained rose rapidly therafter, $P_1 > P_2$, so that by day 20, 80% were P_2 positive. When measured quantitatively, staining intensities were not uniform in all myelin sheaths. Intensity variation was greater with P_2 than with P_1; it decreased with increasing age.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02264-09
LENP

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Animal Models of Neurological Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Sally M. Anderson	Guest Worker	LENP	NINCDS
Others:	Roger Weir	Guest Worker	LENP	NINCDS
	John W. Daly	Chief	LBC	NIADDK
	Carl T. Hansen	Animal Geneticist	AGRC,VR	DRS
	James T. Petras	Neuroanatomist	DNP	WRAIR/WRAMC

COOPERATING UNITS (if any) Laboratory of Bioorganic Chemistry, NIADDK; Animal Genetic Resource, Division of Research Services; Neuroanatomy Branch, Walter Reed Army Institute of Research, Walter Reed Army Medical Center

LAB/BRANCH
Laboratory of Experimental Neuropathology

SECTION
Neurotoxicology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is the investigation of basic mechanisms associated with naturally occurring or artifically neurotoxin-induced neurological diseases through the use of animal models and in vitro experiments. Interactions of various neuroactive drugs and neurotoxins with neurotransmitters in the central nervous system have provided the focus for combined behavioral and neurochemical studies emphasizing basic mechanisms of action of proposed neurotoxins. Two major interests of this project are: A) to define populations of individuals that may be at increased risk to neurological disease resulting from exposure to neurotoxins and B) to use naturally occurring variability in central nervous system function, anatomy and/or neurochemistry, to elucidate mechanisms of actions of neurotoxins. Several different projects have been investigated this year. (1) Interactions of the artificial food color, erythrosin B, with neuronal membranes and neurotransmission have been studied. Erythrosin B has been demonstrated, by several different criteria, to be a potent inhibitor of ATPase activity in brain and other tissues. Its inhibitory potency can be enhanced in vitro by exposing the tissue-erythrosin B complex to light. Studies are in progress to elucidate a possible "ligand-receptor" interaction between ATPases and erythrosin B. (2) We are studying the effects of anticonvulsant drugs on adenosine receptors to promote a better understanding of the actions of convulsant and anticonvulsant drugs. This new information should point out new directions for further biomedical research and lead to therapeutic improvements for these diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02525-04
LENP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Exocytosis Modelling: Kinetics of Membrane Aggregation and Fusion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Stephen J. Morris	Expert	NTS	LENP	NINCDS
Others: Paul D. Smith	Visiting Scientist		BEIB	DRS
Carter C. Gibson	Electronics Engineer		BEIB	DRS
Diane Bradley	Chemist	NTS	LENP	NINCDS
Robert Blumenthal	Section Chief	MSF	LTB	NCI
Anne Walker	Staff Fellow	MSF	LTB	NCI

COOPERATING UNITS *(if any)*

Pharmacology Department, University of Miami Medical School
BEIB, DRS; LTB, NCI

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Neurotoxicology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	0.9	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Neurotransmitter and neuromodulator release takes place by exocytosis; influx of calcium into the cell or nerve terminal triggers the fusion of the storage granule with the cell plasma membrane. These events can be modelled by studying the fusion of artificial or biological membranes with each other. Using a specially constructed stopped-flow mixing apparatus, our previous studies have shown that the kinetics of both aggregation and fusion of small vesicular structures (artificial lipid vesicles, neurotransmitter storage granules, etc) follow second order kinetics with fusion being aggregation rate limited. Our original stopped-flow assay for membrane fusion, based on resonance energy transfer between fluorescent phospholipids was subject to artifacts arising from the interactions of the probes with calcium. We have developed a new assay, based on changes in pyrene-labelled phosphatidyl choline fluorescence which not only solves these problems but allows for rapid, easy calculation of expected results. This assay has been applied to the magnesium-promoted fusion of artificial vesicles as well as protein-catalysed fusion.

A stopped-flow study of cobalt ion transport across the membranes of small unilamellar vesicles shows that they leak profusely during fusion while larger vesicles with less radical changes in surface curvature do not. We ascribe this to defects in the packing structure of the membrane phospholipids. A method for measuring the membrane potential of vesicular structures and changes in potential by various treatments has been derived from this experimental setup.

Various proteins and polypeptides can catalyse fusion of artificial and biological membranes. Some have known functions in biological systems, e.g., the spike proteins from rhabdoviruses; therefore in vitro studies of these fusion mechanisms may have clinical relevance. Polylysine will fuse small unilamellar vesicles under conditions similar to spike protein-mediated virus/cell membrane fusion. Studies indicate that polylysine-mediated fusion is not aggregation rate limited and resembles that seen for in vitro fusion of chromaffin granules.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02550-04 LENP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical and immunologic mechanisms in virally-induced CNS demyelination

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G.L. Stoner	Senior Staff Fellow	LENP	NINCDS
Others:	H.deF. Webster	Chief	LENP	NINCDS
	S.J. Morris	Expert	LENP	NINCDS
	C.F. Ryschkewitsch	Medical Technologist	LENP	NINCDS

COOPERATING UNITS *(if any)*

Department of Biochem., McGill Univ., Montreal, Canada (P.E. Braun)
Department of Medical Microbiology, Univ. of Wisconsin, WI (D. Walker)

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Neurotoxicology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The earlier part of this project focused on myelin basic protein (MBP) structure. Prediction from the known amino acid sequence suggested an organized structure with 25% B-sheet, and challenged the prevailing view of MBP as a disordered protein without long-range intramolecular interactions. This new structure is consistent with an "organizer" role for MBP in myelin, and should lead eventually to an understanding of its structural role. This model is sufficiently detailed to be testable. S.J. Morris in our laboratory has begun fluorescence energy transfer studies which test predictions of the proximity of the naturally fluorescent residues Trp and Tyr. A key feature of MBP is the triproline sequence, the function of which is predicted by the model. The ProProPro sequence follows the sole phosphorylated threonine residue, Thr-99, and appears to be a recognition site for this protein kinase. A search of other known sequences revealed that a similar site occurs in SV40 virus T-antigen, and that the human papova viruses JC and BK have a comparable site with the sequence Pro Lys Thr Pro Pro Pro. The recognition of the common function of these sites in a viral protein and the differentiated product of one of its host cells raises fundamental questions concerning protein evolution, protein kinase identity and specificity, and cross-reactive epitopes. Of primary concern to us are the implications for mechanisms of demyelination. Accordingly, we have examined frozen PML sections for the presence of T-antigen using a sensitive immunocytochemical technique. We detected T-antigen in all 5 cases of PML studied, and found it expressed in many more cells than are virion antigens. We then assessed 9 cases of MS and found no evidence of T-antigen expression. We feel this rules out "enzymological" demyelination in MS in which T-antigen competes for the MBP threonine protein kinase. However, the abundance of T-antigen in infected human PML tissue strengthens the possibility that an immune response to the T-antigen of JC or BK virus plays a role in the etiology of MS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02549-04
LENP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Herpes Simplex Virus Type 2 Infection, CNS Demyelination, and MS.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. R. Martin	Senior Staff Fellow	LENP	NINCDS
Others:	H. deF. Webster	Chief	LENP	NINCDS

COOPERATING UNITS *(if any)*

Department of Ophthalmology, Johns Hopkins School of Medicine (W.R. Green)

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Section on Experimental Neuropathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.8	1.1	2.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects　☐ (b) Human tissues　☒ (c) Neither
　☐ (a1) Minors
　☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project seeks to define determinants of acute CNS demyelination and disease recurrence in experimental herpes simplex virus type 2 (HSV-2) infection, and to refine and test a hypothesis which relates HSV-2 infection to the human demyelinative disease, multiple sclerosis (MS). Our previous studies suggest that major features of HSV epidemiology and pathology are consistent with a hypothesis that HSV-2 is etiologic in MS.

During FY 1985, studies published or in press have provided evidence which further defines the spectrum of experimental disease produced by HSV-2 in the CNS and in lymphoid tissues. These studies provide insights into human infections and disease which HSV-2 is known to cause, and suggests how HSV-2 could produce MS. Specifically, they show for the first time that:

1) When mice are infected by a natural genital route, some animals develop an acute, non-fatal CNS demyelinative disease, while others develop other clinically recognized neurological syndromes, including non-fatal meningitis and fatal panmyelitis. Other mice have no detectable CNS lesions. In man, similar mechanisms could produce non-fatal CNS demyelinative disease in genital HSV-2 infection.

2) Severe infections in mice are accompanied by an acute necrosis of thymic cortical lymphocytes, raising the possibility that severe disease may be due to a virus-induced supression of immunological function. Experiments in newborn mice show that virus can lytically infect some lymphoid tissues (spleen, lymph nodes) but not others (thymus). A direct virus effect on lymphoid tissues is a possible mechanism of immunosuppression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02451-05 LENP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular and Molecular Approaches to Neurotoxicology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Stephen J. Morris	Expert	NTS	LENP	NINCDS
Others: Diane Bradley	Chemist	NTS	LENP	NINCDS
Gerald L. Stoner	Staff Fellow	NTS	LENP	NINCDS
Peter A. Braun	Professor Biochem. Dept. McGill University			

COOPERATING UNITS (if any)

Biochemistry Department, McGill University, Montreal, Que., Canada

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Neurotoxicology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.7	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The calcium-requlated activity of chromaffin cells provide a well-studied system for investigating molecular and cell-surface mediated mechanisms of neurotoxin action. The storage granules of these cells, chromaffin granules, accumulate large concentrations of catecholamines and ATP which are eventually released by exocytosis. Isolated chromaffin granules will aggregate and fuse in the presence of calcium. We have been exploring the molecular basis of these activities. Fluorescent-labelled lipid probes have been successfully inserted into chromaffin granule membranes in vitro without altering the storage properties of the particles. Resonance energy transfer studies of calcium-promoted fusion of these membranes show that, unlike artificial phospholipid vesicles, fusion runs 5-10 fold slower than aggregation. These results support the previous findings that substantial rearrangement of the protein and lipid components of the membrane is required for fusion to occur. This in vitro fusion is inhibited by both organic and inorganic monovalent anions and cations and is insensitive to the presence of Mg-ATP. It is abolished by lysis and resealing the granules.

Neurotoxins are known to disrupt the structure of myelin. Myelin basic protein (MBP), which accounts for 30 percent of CNS myelin proteins, has no known physiological function, although injection of purified MBP will cause experimental Ascending Encephalomyelitis (EAE), considered by some as a model for Multiple Sclerosis. A molecular model for the structure of MBP generates a series of testable predictions. We have been examining the structural properties of MBP using fluorescence and optical spectroscopy. Contrary to many reports, we find evidence for extensive long range structural specificity of myelin basic protein in agreement with the model. These studies may lead to a rapid, more precise functional assay for MBP than induction of EAE.

TAB 5 -- LABORATORY OF MOLECULAR BIOLOGY -- (LMB)

ANNUAL REPORT

October 1, 1984 through September 30, 1985

Laboratory of Molecular Biology
National Institute of Neurological and Communicative
Disorders and Stroke

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Annual Report
October 1, 1984 through September 30, 1985
Laboratory of Molecular Biology
National Institutes of Neurological and Communicative Disorders and Stroke

Ernst Freese, Chief

To align the laboratory's work more with the mission of the NINCDS, most bacterial work has been phased out and only that portion has been retained which either safely yields important results or is needed for the cloning of mammalian genes. Instead, studies in astrocytes on cell differentiation and properties of membrane associated proteins have been emphasized.

1. Control of eukaryotic cell differentiation. Earlier studies by the laboratory had shown that the initiation of bacterial sporulation, which begins when nutrition becomes scarce, is specifically caused by a decrease of GTP. Similar studies in the eukaryotic yeast, Saccharomyces cerevisiae, demonstrated that all types of nutritional deprivation which initiate meiosis and subsequent sporulation cause a decrease in the concentration of both GTP and S-adenosylmethionine (SAM) whereas other nucleotide changes showed no correlation. To determine whether the SAM decrease was important, a double mutant deficient in both GMP and SAM synthesis was constructed and used to show that one can find sporulation conditions (guanine deprivation and limited supply of methionine) under which the concentration of GTP decreases whereas the concentrations of SAM and methionyl-tRNA remain constant. Thus the signal controlling meiosis and sporulation in Bacillus and yeast is GTP. Other researchers had proposed that a decrease of cAMP was important for the onset of meiosis but our lab has clearly shown this not to be case. Experiments assessing the importance of cGMP are underway.

To determine whether deprivation of GTP could induce differentiation also in mammalian cells, glial cells that revealed no glial fibrillary acidic protein (GFAP), by immunofluorescence were used to determine their response to different compounds (such as mycophenolic acid) inhibiting the synthesis of guanine nucleotides. Several cell lines reacted positively by producing astrocyte-like cell extensions and showing GFAP immunofluorescence. This clearly indicated differentiation of these cells. Moreover, when cell proteins were extracted, electrophoresed, transferred to filter paper, and then exposed to anti-GFAP antibody (protein blot), it was surprisingly found that the original cells already produced GFAP and that the amount of protein did not significantly increase during this differentiation. Apparently, the decrease of GTP caused the assembly of GFAP (or increased the sensitivity of cell membranes to alcohol fixation) rather than the production of more GFAP.

These results indicated a serious limitation in using immunofluorescence alone for determining whether glial tumor cells express the GFAP gene. To examine this more thoroughly, cells of benign and malignant gliomas were screened for GFAP expression both by immunofluorescence of cells and by blots of proteins separated electrophoretically by size. So far, four benign gliomas did not express GFAP by the fluorescence assay but did so in protein blots whereas two malignant tumors express GFAP both by fluorescence and by protein blots. Additional cultures are being investigated. Isoelectric focusing of these proteins (separation by charge) followed by staining with anti-GFAP antibody revealed several immuno-reactive proteins of identical molecular weight but different isoelectric points suggesting that the proteins were post-synthetically modified, e.g. by phosphorylation. The GFAP pattern of benign tumors indicated a higher negative charge on the proteins than that of malignant tumors. Apparently, these cells had lost some differentiation step because the pattern of a nine-week old human fetus was identical to that of the malignant tumors. However, the differentiation was not as strong as has been previously claimed since the malignant tumors clearly contained a high proportion of GFAP positive cells.

2. Membrane associated proteins in astrocytes. Because astrocytes serve both structural and nutritional functions in the brain, their plasma membrane containing receptors, transport proteins, and structural proteins plays a particularly important role. The laboratory has investigated several of these membrane-associated proteins. One investigation isolated membrane proteins from brain areas containing "protein assemblies" (Brightman) attached to the membrane and characterized their isoelectric points and molecular weights by two-dimensional electrophoresis. Two particular polypeptides, comprising approximately 10 percent of the total membrane protein, were purified to homogeneity, and sera against them are being raised in rabbits; the major protein was found in astrocytes and neurons but not in kidney cells.

The β-adrenergic receptor protein from C6 glioma cells has been purified 2,000-fold, tagged with a radioactive photoaffinity label, and shown to produce a single spot on two-dimensional electrophoresis. It is now being purified on a preparative scale to determine its amino acid sequence, obtain antibodies, and clone the gene.

A new transport system, driven by a proton gradient across the plasma membrane and maintained by a membrane associated Mg-ATPase has been found in C6 glioma cells and neurons. Certain amines including β-adrenergic antagonists such as propanolol and tricyclic anti-depressants such as imipramine are transported by this system even cells lacking β-adrenergic receptors. The transported compounds can modulate, apparently from within the cell, the responses of both adenylate cyclase and guanylate cyclase to stimulation by neurotransmitters.

3. Comparison of important genes in yeast and astrocytes. Genes coding for proteins important for cellular functions in both lower and higher organisms tend to maintain that sequence information which is essential for the enzymatic or structural function of the protein. The sequence similarity of these genes is often sufficient to enable using the gene from one organism for the isolation of the corresponding gene in another organism by hybridization. Whereas genes in higher organisms can usually be obtained only from cDNA, which does not contain the primary structure of the gene but only reflects its expression as mRNA, it is often possible to obtain the complete gene in yeast. Furthermore, because yeast can be grown in haploid form, mutations in a particular gene can be obtained either by chemical mutagenization or by disruption of the gene with a plasmid. The functional consequences of such mutations and the regulatory mechanisms of such a gene can then be studied more easily than in a diploid eukaryotic cell. The laboratory is investigating two such genes obtained from both yeast and astrocytes.

The first gene is that for glutamine synthetase, which, in the brain, is localized mainly in astrocytes, provides glutamine to neurons for the synthesis of the neurotransmitters glutamate and GABA and serves to detoxify ammonia. In primary cultures of astrocytes, glutamine synthetase activity is inducible by hydrocortisone. Immunofluorescence studies of glial progenitor cells did not reveal the presence of the enzyme whereas it was detected in the derived and more differentiated astrocytes. Thus the study of glutamine synthetase gene expression is likely to reveal a developmental as well as a hormonal regulatory mechanism. In yeast cells, mutants lacking glutamine synthetase activity have been found, and their genetic map location is known. The laboratory has isolated the genes from both a mouse brain cDNA library and from a yeast library. It will compare these genes, make mutations in astrocytes by plasmid insertion into the gene, determine the function of the gene and its control region after transfer from one organism to the other, and study the control mechanism in yeast cells. The other isolated genes concerned with glutamate/GABA metabolism will also be used to determine the appearance of mRNA during astrocyte differentiation.

Nuclear matrix proteins are proteins that remain after the cell nucleus has been exposed to salt, detergent, and DNAse. The laboratory has developed a rapid procedure for isolating both yeast and astrocyte nuclei and obtaining the nuclear matrices free of other cellular material. In two-dimensional gel electrophoresis of the nuclear matrix proteins, about 250 polypeptides were observed using silver staining. A number of monoclonal antibodies against mouse lymphocyte nuclear matrix proteins were used to detect proteins common to yeast and mouse astrocytes. One such protein was observed and the corresponding antibody was employed to isolate the gene from a yeast and a mouse cDNA library. This isolated gene will now be used to mutate the corresponding chromosomal gene in yeast in order to determine its functional role in mating, mitosis, and meiosis. The gene will also be used for in situ hybridization to determine at what stage of embryonic development the gene for this nuclear matrix protein starts to be expressed.

4. Analysis of a cloned operon expressed only during development. It is generally not known how cells control the expression of developmental genes on the molecular level. We have investigated the gene for glucose dehydrogenase which is expressed in Bacillus subtilis only during differentiation and is then found only in one cell comparment (forespore). The nucleotide sequence of this gene and its flanking DNA has been determined. The structural gene contains 700 base pairs and is preceded by another open reading frame (855 base pairs); the two structural genes together comprise a sporulation specific operon. The nucleotide sequences of the ribosome binding and putative promotor sites have been identified. This control region, which is located in an 0.5 kb DNA fragment, also contains the DNA area important for the regulation of the operon during differentiation because glucose dehydrogenase is expressed constitutively when this region is removed. This promotor region is now being transferred to the head of structural genes and or is being mutated to determine the mechanism whereby the expression of the glucose dehydrogenase gene is limited to differentiating cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02677-01 LMB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Differentiation and Regulation of Gene Expression in Glial Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

L. Vitkovic, Senior Staff Fellow, LMB, NINCDS
R. H. Lipsky, Staff Fellow, LMB, NINCDS

COOPERATING UNITS (if any)
Section on Structural Plasticity, LNB, NINCDS (M. Brightman)
Institute of Neurology, University of London (M. Noble)

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Developmental Biology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.7	4.0	0.7

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Astrocytes' differentiation and function are investigated at protein and gene levels. To identify astrocyte-specific markers, plasma membrane, nuclear, and nuclear matrix proteins were resolved by two-dimensional electrophoresis and compared to those in neurons, glioma C6 cells, and kidney fibroblasts. Two abundant membrane proteins were identified, purified to homogeneity, and will be characterized by means of antibodies. To characterize evolution of brain gliomas, we examined expression of glial fibrillary acidic protein (GFAP), an astrocyte marker, in normal and transformed human astrocytes. In these cells GFAP appeared to be regulated by post-translational modification and its assembly seemed to be controlled by GTP. GFAP in malignant gliomas and fetal astrocytes were similarly charged, while in benign glioma cells it was more electronegative.

Brain glutamine synthetase, a key enzyme in nitrogen metabolism and localized in astrocytes, is regulated developmentally and hormonally. Five cDNA clones were identified in a mouse brain library that hybridized with a cloned hamster glutamine synthetase gene. We will use these clones to study the transcriptional regulation of the glutamine synthetase gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02680-01 LMB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Nucleotide Sequence and Control of the GlcDH Operon

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

K. A. Lampel, Senior Staff Fellow, LMB, NINCDS

COOPERATING UNITS (if any)

Dr. R. Ramaley, University of Nebraska Medical Center, Omaha, NE
Prof. P. Fortnagel, Institute für Allgemeine Botanik, West Germany
Dr. N. Vasantha, Genex Corporation, Gaithersburg, MD

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Developmental Biology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.9	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
  ☐ (a1) Minors
  ☐ (a2) Interviews
☐ (b) Human tissues
☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During the differentiation (sporulation) of Bacillus subtilis many new proteins with developmental functions are made. Because it is generally not known how the synthesis for developmental proteins are regulated, we have investigated one of them, glucose dehydrogenase and its developmentally-regulated gene (gld) in detail. The nucleotide sequence of the gld gene and the flanking DNA has been determined. The glucose dehydrogenase structural gene (780 base pairs) and an open reading frame (855 base pairs) that precedes it comprise a sporulation-specific operon. The latter is an essential gene for differentiation because its deletion from the Bacillus subtilis chromosome prevents the cell from sporulating. The nucleotide sequences of the ribosome binding and putative promoter sites have been identified. The promoter and regulatory region of the operon are located in a 0.5 kilobase (kb) PvuI fragment of plasmid pEF1 which was constructed by inserting a 4.0 kilobase EcoRI DNA fragment containing the structural gene of glucose dehydrogenase into the Escherichia coli plasmid, pBR322. Preceding the glucose dehydrogenase operon is another gene that is expressed coordinately with the gld gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	**PROJECT NUMBER** Z01 NS 01244-21 LMB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Control Mechanisms and Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

E. Freese, Chief, Laboratory of Molecular Biology, NINCDS
S. Silverman, Senior Staff Fellow, LMB, NINCDS

COOPERATING UNITS *(if any)*
Biomedical Institute, University of Freiburg, West Germany (Prof. H. Holzer)
Medicine Branch, National Cancer Institute (Dr. Lippman)

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Developmental Biology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	4.3	0.7

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The switch from mitosis to meiosis and sporulation in the yeast Saccharomyces cerevisiae, which is normally initiated by nutritional deprivation, can be specifically initiated by partial guanine or methionine deprivation. All these conditions lead to the decrease of intracellular GTP whereas the concentrations of other nucleotides remain constant, increase or decrease, depending on the particular condition used to initiate meiosis. Involvement of other metabolites in sporulation induction has been ruled out; in particular, conditions can be found under which the cellular GTP decreases while that of S-adenosylmethionine or cyclic AMP does not change and yet the cells go through meiosis and sporulate well. Nuclear and nuclear matrix proteins where subjected to two-dimensional electrophoresis and certain proteins were found to change during meiosis. The gene for one nuclear matrix proteins was cloned from both a mouse and a yeast DNA library. The gene for glutamine synthetase was similary obtained from these two libraries. In Bacillus subtilis partially inhibitory concentrations of ethionine caused continual sporulation in eth mutants. This effect depends on the conversion of ethionine to S-adenosylethionine and presumably involves the inhibition of a methylation reaction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02365-07 LMB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cellular Responses to Hormones and Neurotransmitters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

R.C. Henneberry, Chief, Molecular Neurobiology Section, Laboratory of Molecular Biology, NINCDS

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Molecular Neurobiology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our objective is to elucidate how mammalian cells perceive and respond to the signals in their environment. We have studied the effects of hormones and neurotransmitters which bind to and remain at extracellular receptors while transmitting information across the membrane. We have purified the beta-adrenergic receptor 2264-fold from C6 glioma cells, tagged the protein with a radioactive photoaffinity label, and demonstrated a single spot on autoradiographs of 2-D gels. Currently, we are purifying the receptor on a preparative scale to establish the amino acid sequence, clone the gene, and generate molecular probes to study regulation of receptor synthesis. Separate from receptors, we have found a new active transport system for a category of amines which includes beta-adrenergic antagonists such as propranolol and tricyclic antidepressants such as imipramine; transport is driven by a proton gradient across the plasma membrane maintained by a membrane-associated mgATPase. Transport by this system is fully active also in cells lacking beta-adrenergic receptors. Following the transportation and accumulation inside neurons and glial cells, tricyclic antidepressants such as imipramine can modulate the responses of both adenylate cyclase and guanylate cyclase to stimulation by neurotransmitters.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02527-04 LMB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Role of Methylation in Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

E. Freese, Chief, Laboratory of Molecular Biology, NINCDS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Developmental Biology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Part of this project is now included in Project Number Z01 NS 01244-21 LMB.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01886-15 LMB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Control of Meiosis and Morphogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

E.B. Freese, Biologist, LMB, NINCDS
S. Silverman, Senior Staff Fellow, LMB, NINCDS

COOPERATING UNITS (if any)
NONE

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Developmental Biology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects
  ☐ (a1) Minors
  ☐ (a2) Interviews
☐ (b) Human tissues
☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Part of this project is now included in Project Number Z01 NS 01244-21 LMB.

PHS 6040 (Rev. 1/84)

GPO 914-918

TAB 6 -- LABORATORY OF MOLECULAR GENETICS -- (LMG)

ANNUAL REPORT

October 1, 1984 through September 30, 1985

Laboratory of Molecular Genetics

National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT
October 1, 1984 through September 30, 1985
Laboratory of Molecular Genetics
National Institute of Neurological and Communicative Disorders and Stroke

Robert A. Lazzarini, Chief

1985 was a watershed year for the Laboratory of Molecular Genetics. The year marked the transition from the developmental to the investigative stages for the research programs concerned with the developmental program of oligodendrocytes. The necessary materials were obtained, the new techniques devised and refined, and the stage set for the rapid exploration of the cell biology of the oligodendrocyte. With regard to its molecular programs, new, exciting developments have propelled them to the very forefront of the field. These programs are well into the exploratory phase and are yielding big dividends. The year also marked the completion of several virus programs which had been the main staple of the laboratory during its very early days. The successful completion of these programs and the initiation of new programs concerned with degenerative neurologic phenomena seen in Alzheimer's, Scrapie, and Creutzfeldt-Jakob diseases have shifted the balance between virus research and neurobiological research of the laboratory. Currently, virus research accounts for only about 25% of the research effort. We anticipate that it will remain at this level in the foreseeable future.

Our studies on the differentiation of the myelin forming cells made a quantum leap this year. We have now perfected conditions for in situ hybridization of both cultured oligodendrocytes and frozen sections of whole brain. We have devised ways of performing double label experiments in which fluorescent microscopy using fluorescein conjugated specific antibodies and in situ hybridization can be performed on the same specimens. With these tools, we have studied the emergence of myelin basic protein (MBP) mRNA, and protein in developing oligodendrocytes in vivo and in vitro. Much to our surprise, the emergence of MBP expression in cultured oligodendrocytes follows the same timetable as observed in the animal, suggesting that the timing of myelin synthesis is determined very early in fetal life. Oligodendrocytes removed from new born mouse brains are devoid of myelin basic protein, mRNA, and protein, but after six days in culture, MBP gene expression is evident. This is precisely the same time that oligodendrocytes first express these genes in the intact mouse.

We have employed in situ hybridizations to study the remyelination of plaque areas of the brain which develop in response to disease. In mice infected with mouse hepatitis virus, demyelination is extensive and followed by remyelination as a normal progression of the disease. By in situ hybridization, we were able to identify oligodendrocytes at the periphery of the plaque area which were synthesizing large amounts of myelin basic protein mRNA. Interestingly, oligodendrocytes at some distance from the plaque were also stimulated to express MBP and, in general, there appeared to be a gradient of expression of MBP; highest at the periphery of the plaque and diminishing with distance away from the plaque. In preliminary experiments, we have examined multiple sclerosis (MS) brain for evidence of stimulation of MBP gene expression near plaques. Our results clearly demonstrate that oligodendrocytes near the plaque area of multiple sclerosis brains are stimulated to produce large amounts of MBP mRNA. However, remyelination in MS is very limited and, therefore, we are investigating whether remyelination of plaques is initiated, but not completed in MS patients.

The interaction between the neuron and the differentiating oligodendrocyte was also investigated with video-enhanced, differential interference contrast microscopy in a time-lapse mode. The motile activities displayed by oligodendrocytes in culture seem to be characteristic of the cell and may play an important role in enabling the oligodendrocyte to find the target axon and in transporting myelin membrane components to the site of myelin assembly.

The molecular level studies of myelin gene expression have developed exponentially. One of the curious facets of MBP expression is that there are four forms of MBP which differ in amino acid sequence. Our studies on the structure of the MBP gene has proven that these four forms of myelin basic protein are synthesized from one gene, not four, by a mechanism which involved alternative modes of splicing of the large primary transcript of this gene. We have mapped the various exons in the gene and have studied the exon and intron boundaries and splicing pattern. Other results suggest that alternative splicing may be very common in the central nervous system (CNS).

Our efforts at cloning other oligodendrocyte specific mRNAs has been quite successful. We now have full-length clones of proteolipid protein, human myelin basic protein, human myelin associated glycoprotein (MAG), and cyclonucleotide phosphohydrolase (CNP), and several unidentified oligodendrocyte specific cDNAs. Our current efforts are directed towards studying the control elements of these genes in order to understand the mechanisms by which these genes are simultaneously turned on during differentiation.

Three aspects of virus-host cell interactions have been studied this year. In the first, we ask the question: What elements of the virus determine its cell tropism -- that is, determine which cell will be infected by the virus? Our evidence suggests that the viral envelope glycoprotein plays a pivotal role in determining cell tropism. We have cloned the surface glycoproteins of measles virus and sequenced our cDNA clones in order to deduce the amino acid sequence of the glycoproteins. The deduced sequences have revealed several interesting properties of these two glycoproteins. The fusion protein undergoes a proteolytic cleavage to yield to fractions F_2 and F_1, and we now know the sequence of this cleavage site. We have identified the carboxyl terminal hydrophobic domain which serves to anchor the fusion protein in the virus membrane. We have identified four putative glycosylation sites, all in the F_2 portion of the protein. With respect to the HA protein, the membrane insertion site is at the amino terminus of the protein and is 24 amino acids long. There are five glycosylation sites within a block of 50 amino acids. We now intend to clone and sequence the fusion and HA proteins from neurotropic strains in order to complete our comparison of neurotropic with wild-type measles virus.

In the second viral program, we have undertaken the study of the massive polymerase complex which synthesizes both genomic and messenger viral RNAs. We have cDNA cloned and sequenced the entire gene specifying this massive protein.

By splicing together several clones, we have assembled a full-length cDNA clone. We have inserted this clone into an expression and have been able to demonstrate that the protein expressed by this gene is a functional polymerase which is able to complement and rescue conditional polymerase mutants of Vesicular stomatitis virus. This unique system will allow us to identify and dissect the functional domains of the polymerase protein and, eventually, to fully understand the replication of negative strand RNA viruses.

The third and final viral program concerns the mechanism and the process of viral assembly in the infected cell. Using high resolution views of platinum replicas of the plasma membrane of infected cells, we have been able to identify various stages of viral assembly. Combining this approach with immunostaining using immunogold labeling, we have determined how the nucleocapsid of VSV interacts with the viral glycoprotein which is integrated into the plasma membrane and how the M protein induces the helical coiling of the viral nucleocapsid.

Finally, although not specifically addressed yet in specific project reports, we have initiated a study of the paired helical filaments that are found in certain degenerative diseases of the CNS: Alzheimer's, Scrapie, Creutzfeldt-Jakob, and in brains of aged individuals and Down's syndrome patients. As a first step, we have obtained clones of the human neurofilament mRNA and are in the process of sequencing these clones to determine the amino acid sequence of the small subunit of human neurofilaments. In parallel, we have devised oligonucleotide probes for the gene encoding one protein found in the neurofibrillary tangles. Similarly, we have synthesized oligopeptides that correspond to a portion of the protein found in neurofibrillary tangles and have raised antibodies to that peptide. Both of these probes will be employed to identify cDNA clones of the gene coding for the neurofibrillary proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02528-04 LMG

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Myelin Synthesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. A. Lazzarini	Chief	LMG, NINCDS
Others:	L. Hudson	Senior Staff Fellow	LMG, NINCDS
	J. Kamholz	Medical Staff Fellow	LMG, NINCDS
	C. Puckett	Medical Staff Fellow	LMG, NINCDS
	D. Nelson	Staff Fellow	LMG, NINCDS
	S. Molineaux	Guest Worker	LMG, NINCDS
	F. de Ferra	FIC Visiting Fellow	LMG, NINCDS
	B. Lewis	Biological Lab Technician	LMG, NINCDS

COOPERATING UNITS *(if any)*

Department of Biology, University of Maryland

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Recombinant Genetics Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8	7	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Four proteins of a peripheral and central nervous system have been targeted for study -- the myelin basic protein, proteolipid, myelin associated glycoprotein, and cyclic nucleotide phosphohydrolase. The first phase of the molecular level studies is the cloning of the genes coding for these proteins. To this end, we have obtained the necessary human perinatal brain tissue, prepared cDNA libraries from brain mRNAs, and have searched among the five million library clones in order to identify those which contain the genes for myelin basic proteins. We have positively identified several clones of each protein and are characterizing them extensively.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02026-13 LMG

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Viral Nucleic Acid Synthesis in Animal Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Schubert	Research Chemist	LMG, NINCDS
Others:	E. Meier	Visiting Fellow	LMG, NINCDS
	G. Harmison, II	Chemist	LMG, NINCDS
	L. Hudson	Senior Staff Fellow	LMG, NINCDS

COOPERATING UNITS (if any)

Sue Emerson, University of Virginia, Charlottesville, VA

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Molecular Virology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The RNA dependent RNA polymerase of negative strand RNA viruses transcribes and replicates the viral genome. Despite the fact that many members of these viruses cause diseases of the central nervous system, little is known about the RNA polymerase and the multiple enzymatic functions it performs. Since many of these functions are essential to the virus and differ from those of the host cell, they represent potential targets that could specifically be inactivated without affecting cellular mechanisms. Therefore, the study of these viral specific mechanisms could be exploited in the treatment or prevention of viral infections in the central nervous system.

In order to study the multiple functions of the polymerase complex, we have cDNA cloned, sequenced, assembled and expressed a functional 241 kilodalton polymerase protein of Vesicular stomatitis virus (VSV). We have currently established a eukaryotic cell line which constitutively expresses the polymerase protein. Functionality of the polymerase was demonstrated by complementation and rescue of conditional polymerase mutants of VSV.

Hence, this unique system will allow for the first time the identification and dissection of the functional domains of the polymerase of negative strand viruses through site specific mutagenesis. The effect of these mutations on the mutation rate of the polymerase, itself, will also be studied with respect to the establishment and maintenance of persistent infections. Towards these ends, we have generated over 20 mutant recombinant L genes and are currently studying their effects on the performance of the multiple functions of the polymerase complex.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02600-03 LMG

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Assembly of Enveloped RNA Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Dubois-Dalcq	Section Chief	LMG, NINCDS
Others:	C. Jordan	Staff Fellow	LMG, NINCDS
	W. Odenwald	Microbiologist	LMG, NINCDS
	K. Ono	FIC Visiting Fellow	LMG, NINCDS
	R. Rusten	Biological Lab Technician	LMG, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Molecular Virology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.3	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The elucidation of mechanisms of synthesis, processing and transport of viral components at the molecular level will help us to understand mechanisms that govern biosynthesis of normal cellular components and to understand problems of defective viral assembly implicated in some neurological diseases. To study the biosynthesis of viral macromolecular components, we have prepared a battery of monoclonal antibodies reacting with different sites of polypeptides of two negative stranded RNA viruses (Vesicular stomatitis virus (VSV) and measles virus), polyclonal antibodies made against synthetic peptides corresponding in sequence to portions of the viral polypeptides, and genes coding for some of the viral polypeptides of the Vesicular stomatitis virus cloned into convenient expression vectors.

One part of the project concerns the study of the ultimate assembly of viral components at the sites of viral budding. High resolution views are obtained from platinum replicas of the outer and inner side of the plasma membrane of cells infected with VSV. Combining this approach with the immunogold labeling technique for viral proteins, we determine how the nucleocapsid interacts with the viral glycoprotein integrated in the plasma membrane and how the M protein may induce nucleocapsid coiling during viral maturation. Studies with a temperature-sensitive mutant in the M protein of VSV indicate that M protein normal transport to the membrane and normal conformation is necessary for nucleocapsid coiling and viral budding to occur. Another part of the project is to analyze the function of the non-structural protein C of measles virus by microinjection of living cells with specific antibodies into this protein.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02580-03 LMG

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Determinants of Viruc-Host Cell Tropism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. J. Bellini	Special Expert	LMG, NINCDS
Others:	C. Richardson	Special Expert	LMG, NINCDS
	S. Rozenblatt	Visiting Associate (8/83-8/84)	LMG, NINCDS
	G. Englund	Biologist	LMG, NINCDS

COOPERATING UNITS *(if any)*

Neuroimmunology Branch, NINCDS and Dr. Bert Rima, Department of Biochemistry, The Queen's University of Belfast, Belfast, Northern Ireland.

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Molecular Virology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has as a final objective, the elucidation of those viral and host cell components which influence, at the molecular level, the phenomenon known as viral tropism. Currently, the emphasis of this project is focused on those components of measles virus which pertain to the neurotropism of this clinically relevant paramyxovirus.

During the course of natural infection, neurotropic variants of measles virus are generated. Frequently, this gives rise to mild central nervous system involvement and, less frequently, to clinical encephalitis. In rare instances, a delayed encephalitis, subacute sclerosing parencephalitis, is observed. Although the mechanism(s) of this neurotropism is unknown, available evidence suggests that the viral envelope glycoproteins are involved and can be antigenically distinguished from the wild-type or vaccine strains using hybridoma antisera. Therefore, the initial phase of this project is to clone those genes encoding these proteins from a vaccine strain (Edmonston) of measles virus. From the nucleotide sequence, we will deduce the amino acid sequence of the proteins. To positively identify these clones, oligopeptides from the deduced amino acid sequence will be synthesized. Antisera raised against the synthetic peptides will then be used in a variety of immunologic techniques to identify the viral protein recognized and, thus, assign the clones. Once this is established, fragments of these cDNA clones will then be used as probes to identify their counterparts in neurotropic strains of measles virus presently available in our laboratory. The nucleotide and deduced amino acid sequence of the glycoproteins of the neurotropic strains will then be compared with the vaccine and wild-type virus for regions of homology and non-homology. The cloned glycoprotein genes will be placed in appropriate expression vectors to permit the study of their synthesis, regulation of expression, maturation and insertion into the host cell membrane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02034-13 LMG

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biology of Myelin-Forming Cells In Vitro and In Vivo

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Dubois-Dalcq	Section Chief	LMG, NINCDS
Others:	N. Zeller	Senior Staff Fellow	LMG, NINCDS
	K. Kristensson	Guest Scientist (11/84-4/85)	MS Society
	T. Behar	Microbiologist	LMG, NINCDS
	R. Rusten	Biological Lab Technician	LMG, NINCDS

COOPERATING UNITS (if any)

K. V. Holmes, Professor of Microbiology, Department of Pathology, USUHS; D. Pleasure, Professor of Neurology, University of Pennsylvania; B. Kachar, Visiting Associate, Laboratory of Neurobiology, NINCDS.

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Neural and Molecular Ultrastructure Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.7	2.5	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Myelin sheath is essential to normal conduction in nerves and is altered in multiple sclerosis and Guillain-Barre diseases. Understanding how myelin is formed and repaired requires basic studies of the differentiation of myelin-forming cells both in vitro and in vivo. To this aim, we are culturing myelin-forming cells in isolation, obtaining enriched populations, and studying the differentiation of these cells. Oligodendrocytes cultured without neurons develop on schedule a complex phenotype similar to their in vivo counterpart. Although devoid of intermediate filaments, they display specific motile activities which may function in vivo to find the target-axon and transport myelin components at the site of myelin assembly. Moreover, these cultured cells express MBP, MAG, and PLP in a predetermined sequence and timing, independent of continuous neuronal influences. MBP specific mRNA is found in the cell body and is immediately followed by the appearance of MBP, suggesting a control of gene expression at the level of transcription. In vivo, the developmental expression of MBP message in different areas of the CNS closely follows the emergence of MBP protein as detected by immunocytochemical method. During demyelination caused by a coronavirus in mice, the in situ hybridization method is used to detect new synthesis of myelin specific messages around the lesion and appears to be the ideal tool to analyze the reactivity of oligodendrocytes to the disease process. In order to elucidate mechanisms of myelin formation, defects in myelin assembly are being analyzed in a rat genetic mutant.

TAB 7 -- LABORATORY OF NEURAL CONTROL -- (LNLC)

ANNUAL REPORT

October 1, 1984 through September 30, 1985

Laboratory of Neural Control, Intramural Research Program
National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT
October 1, 1984 through September 30, 1985
Laboratory of Neural Control, Intramural Research Program
National Institute of Neurological and Communicative Disorders and Stroke

Robert E. Burke, M.D., Chief

Introduction

Research work in the Laboratory of Neural Control (LNLC) is devoted largely to studies of the central and peripheral neural mechanisms involved in the control of movement in mammals, emphasizing neural organizations at the level of the spinal cord and those regions of the brain stem and cerebral cortex that project directly to the spinal cord.

Present Organization

During FY 1985, the staff of the Laboratory of Neural Control (LNLC) included 13 professional scientists (four permanent senior scientists and nine post-doctoral fellows). The permanent staff also includes three senior support personnel (two engineers and one physiologist), a biological technician, and one laboratory secretary. Non-permanent staff includes three graduate students, one computer programmer, one engineering aide, one laboratory aide, and one Junior Fellow. Because of the close interaction and collaboration among the Laboratory staff, LNLC has not been divided into formal Sections. The research effort can be described under four general headings, divided roughly by methodological approach:

1. Electrophysiological and morphological analysis of the cellular physiology and neuronal circuitry operating in the control of movement at the spinal cord level, largely using acute, reduced preparations (primarily cats).

2. Projects that utilize novel methods for recording the activity of individual neural elements, activity patterns in whole muscles, and kinesiological data in awake, intact animals (both cat and monkey) that are comfortable and performing normal motor behaviors.

3. Theoretical and computer modeling studies of: a.) the electrophysiological properties of identified central nervous system neurons; b.) information processing in neural networks; c.) the mechanical arrangements of bones, joints and muscles in the cat hindlimb with a view to providing a comprehensive description of their dynamic actions; and d.) the properties of complex elements such as muscle spindles.

4. Activities concerned directly with the development of new instruments and techniques, and the further refinement of existing methods, for recording and analyzing neurally-relevant data from intact, freely moving animals and for computer-assisted reconstruction of the anatomy of functionally identified neural elements.

Project Summaries:

Many of the projects underway in LNLC are highly integrated and interactive with one another. The following summaries deal with major points under individual headings but points of overlap will be apparent.

Motor Control Systems in the Spinal Cord: During FY 1985, we completed a long-range project concerning estimation of passive membrane properties in type-identified α-motoneurons and of the factors that control the shape and amplitude of group Ia excitatory postsynaptic potentials (EPSPs) in these cells. The experimental data base and main methods for these studies has been discussed in previous Annual Reports. This work concentrated on completing computer model studies of EPSP generation in alpha-motoneurons with realistic anatomy and best-fit estimates of passive membrane characteristics. Anatomical distributions of active synapses on the model neurons were based on the spatial probability of occurrence of group Ia synapses in earlier work.

The results of these synaptic modeling studies show that the composite EPSPs produced in model motoneurons have amplitudes and shapes that match very well those observed experimentally in cat motoneurons in this and other laboratories. These studies suggest that the factors that control synaptic potential amplitude in motoneurons, and presumably in neurons generally, involve a complex interaction between the number and spatial distribution of active synapses, in conjunction with the postsynaptic characteristics of the recipient neuron (membrane area, dendritic geometry, and membrane resistance and capacitance). In the case of motoneurons and group Ia synapses, the available evidence suggests that the main factors that control Ia EPSP amplitude are synaptic density and the dendritic/somatic conductance ratio.

In addition, we have examined the shapes and amplitudes of EPSPs produced by spatial distributions of synapses that derive from individual group Ia afferents to individual α-motoneurons. Despite considerable anatomical dispersion in such contact systems, the shapes of EPSPs they generate usually showed no evident components with different electrotonic location. The shape indices (rise time and half-width) of these "single fiber EPSPs" resembled those obtained experimentally in cat motoneurons. When postsynaptic membrane resisitivity is non-uniform, our studies indicate that the relation between EPSP amplitudes and shapes at the cell soma, and the electrotonic location of relevant synapses, is considerably more complex than suggested by earlier equivalent cylinder models. These studies have provided new insights into the factors that control the flow of synaptic currents in complex dendritic networks that were not apparent in previous studies of idealized model systems.

We have continued to examine the organization of excitatory interneuronal pathways to motoneurons in the cat spinal cord, with emphasis on the cutaneous input pathways that project to motoneurons of the flexor digitorum longus (FDL) nucleus. The major aim is to characterize, for the first time, sets of functionally-identified excitatory segmental interneurons that directly excite alpha motoneurons. To date, the three classes of segmental interneurons that have been functionally identifed are all inhibitory to motoneurons. Further progress in several areas, including elucidation of the circuitry in the segmental central pattern generator (CPG) for locomotion, depends on additional information about the sets of interneurons that directly excite motoneurons.

In order to identify and characterize such "last-order" interneurons, axonal destination (i.e., direct projection to α-motoneurons) must be demonstrated. This is practical only when there are a variety of initial clues to identify candidate interneurons for detailed testing. This is most readily accomplished for interneurons in disynaptic reflex pathways, where the target interneurons receive direct (monosynaptic) input from defined sets of primary afferents and in turn project directly to specific groups of alpha motoneurons. Most excitatory reflex pathways in the cat spinal cord have been thought to be trisynaptic (i.e., contain two interneurons between afferents and motoneurons), which greatly complicates analysis. However, a high proportion of FDL motoneurons receive excitatory synaptic input from distal skin regions of the ipsilateral limb at central latencies (less than 1.8 ms) that suggest disynaptic connectivity. During FY 1985, we extended our data set and showed that this cutaneous pathway receives excitatory convergence from the rubrospinal and pyramidal systems. Such patterns of input convergence serve to identify segmental interneurons that could be members of the target set.

In addition, FDL motoneurons exhibit a distinctive activity pattern during locomotion in the intact cat, which is preserved during "fictive locomotion" in the decerebrate, paralyzed cat preparation. The distinctive timing of FDL activity during fictive locomotion is presumably driven by a specific set of excitatory interneurons that can be recognized by their firing pattern during fictive locomotion. Our current working hypothesis is that these interneurons are identical with those that convey disynaptic cutaneous excitation to FDL motoneurons. Preliminary evidence shows that transmission of oligosynaptic excitation from distal skin to FDL motoneurons is enhanced during postsynaptic depolarization waves that produce the distinctive flexion bursts in FDL cells during fictive stepping. This supports our working hypothesis but additional evidence will be needed to rule out alternative explanations. Investigation of this question will be a major effort in FY 1986.

Intrinsic Properties of Motor Units: Further analysis of our extensive data set on the detailed anatomy of fully-reconstructed triceps surae alpha-motoneurons of identified motor unit type has shown significant differences between the dendritic trees of cells that innervate fast versus slow twitch muscle units. Of particular interest is a clear difference in the branching topology of the two cell groups, which presumably arise during development. The topology of binary branching trees is a field of considerable theoretical interest to several disciplines. We will continue to examine this subject with reconstructions of cells belonging to other motor nuclei and of labeled gamma motoneurons.

Characterization of the motor unit population in the cat tenuissimus muscle has continued in collaboration with an investigator at the Hebrew University, Jerusalem, Israel. Work at NIH is designed to elucidate the physiology, histochemistry, and morphology of tenuissimus motor units, while the characteristics of neuromuscular transmission in type-identified muscle units is being done in Israel. This project offers, for the first time, an opportunity to study neuromuscular junctional mechanisms in different types of muscle fibers within the same mammalian skeletal muscle.

The project entitled "Neuromuscular Coordination of Movement" includes a variety of studies that utilize both novel and conventional experimental methods to study motor performance in intact, behaving cats. The new methods primarily involve chronically-implanted transducer systems developed and perfected in LNLC. The motivating philosophy in this work is to obtain information from intact, freely behaving animals in a form that enables interpretation according to the very large data base accumulated about the behavior of neural elements in anesthetized, immobilized, or otherwise reduced preparations. Much of the current work in this project is closely related to that in the project entitled "Models of Neural Interactions" and the two can be summarized together. These projects also interface importantly with the two projects already discussed above.

During FY-85, we have continued a detailed analysis of the cat hindlimb musculature with respect to the anatomical interrelations between muscles and muscle groups in relation to their functional activity. Of particular interest are structure-function correlations in bi- or multi-articular muscles that exhibit different functions, depending on limb and/or body positions during normal movements. Insights gained in hindlimb muscles are being further explored in the highly complex muscles that control head and neck movement in the cat, in collaboration with a research group at Queen's University in Kingston, Canada. We continue to refine the data base underlying a computer-based model of cat hindlimb musculature being developed under contract with the Department of Electrical Engineering at the University of Maryland, College Park. In these studies, we are able to take into account the Newtonian dynamics of moving limb segments in relation to the active shortening and active lengthening of multiarticular muscles, which are almost impossible to deal with in any other way. The model includes detailed descriptions of muscle anatomy, including internal architecture and average sarcomere lengths. We are engaged in developing a laser diffraction system to permit direct estimates of sarcomere length in target muscle in situ. These studies continue to provide important insights which guide further experiments on the existence and function of "task groups" of motor units and their associated synaptic inputs. The task group hypothesis provides a unifying concept for detailed analysis of the neural control of moving limbs in actual motor behaviors.

Several aspects of microanalysis of motor control are also ongoing. For example, a number of muscles are composed of muscle fibers arranged in series. This provides for effective force production over much longer ranges of length change than are possible with parallel or pinnate muscle fiber architecture but the series arrangement introduces serious problems for neural control. In such muscles, the muscle fibers in individual muscle units must be spatially organized in order to provide for effective force transfer from origin to insertion. In addition, there must presumably be mechanisms within the spinal cord to ensure appropriate activation of motor units into efficient task groups. The anatomical and functional solutions to this problem are quite unclear and are being studied in serial muscles like the tenuissimus and sartorius, as well as in certain neck muscles. Another aspect of microanalysis concerns continued study of the gating of transmission of afferent information ("reflexes") during normal and fictive locomotion and scratching. Emphasis in FY 1985 has been on analysis of presynaptic modulation of afferent input to the spinal cord. Surprisingly, there are sufficient levels of presynaptic depolarization (usually associated with "presynaptic inhibition") to produce dorsal root reflexes in extensor muscle afferents during the flexion phase of stepping in intact cats, as well as in decerebrate locomotion.

Work on "Cortical Mechanisms of Voluntary Motor Control" has, during FY 1985, continued to examine the organization of motor output regions of the primate motor cortex during the performance of voluntary movement in awake monkeys. The discharge patterns from individual neurons in the arm/hand area of the cerebral cortex that have relatively direct pathways to the spinal cord and brain stem (the sensorimotor cortex and supplementary motor area) are recorded during movement performance in minimally restrained, alert monkeys. Recent results have emphasized the importance of monitoring the electrical (EMG) activity in multiple forelimb muscles during recording of discharge patterns or intracortical microstimulation (ICMS).

We have continued to compare the results of ICMS with the activation patterns of cortical neurons recorded at the same points during voluntary movement, in order to assess the role of particular groups of motor cortical neurons on alternating versus co-contraction patterns of activation of agonist and antagonist muscles. The organization of cortical inhibition, presumably operating through spinal segmental interneuron systems, is of particular interest, since we have found that zones that produce inhibition of target muscles often border, or surround, zones that produce pure excitation. Much of this complexity had been missed in other studies of cortical organization because multi-muscle EMG methods were not utilized. Extensive maps of cortical areas associated with excitation or inhibition of particular muscles have been prepared. These show that individual muscles are affected by cells within extensive cortical regions, which often exhibit overlap with regions associated with other muscles or muscle groups. These results strongly suggest that the topography of cortical representation is organized in terms of movement patterns rather than individual muscles. Observations on cortical cell discharge patterns during small mechanical perturbations of the manipulandum during practiced movements suggests that the much-debated "long-loop" reflexes may in fact be present in intact animals, and thus must be taken into account in theories of cortical motor control. Preliminary trials have begun to utilize nuclear magnetic resonance scanning to better localize the cortical areas associated with hand and arm movement control.

The flexor carpi ulnaris (FCU) motor pool is of particular interest in this project because this muscle is composed with two distinct heads with very different histochemical muscle fiber compositions. In preparation for detailed studies of recruitment sequences in the two heads, the motor pools of the two heads have been labeled by retrograde transport of horseradish peroxidase. Surprisingly, the motoneurons supplying the two heads are coextensive within the same motor cell column in the C8 to T2 spinal segments. If the two heads are used differentially, motoneuron control must be differentially distributed without reference to intra-spinal topography.

Finally, collaborative work in this project has been initiated to assess several forms of multi-lead intracortical recording electrodes, fabricated by outside sources using thin-film technology for evaluation as tools for both research and possible clinical application in neural prostheses. A number of designs have been studied but, unfortunately, each has exhibited particular problems related to the materials used or the techniques of insertion. This evaluation will continue because multi-lead electrodes offer significant promise for solving certain questions in both basic and applied research areas.

Work done under the project entitled "Techniques for Making Contact with the Nervous System" largely results from requirements for special instrumentation generated by other projects in LNLC. Because many of the techniques and instruments developed in LNLC are new and without counterpart commercially, LNLC staff attempt to provide assistance to other scientists at NIH and at other institutions around the world who request information and advice about specific data acquisition and processing problems.

During FY 1985, we completed software development for a computer - microscope interface system, which is designed to facilitate collection of quantitative data about neuronal morphology. The hardware has been described in previous Annual Reports. Two major reconstruction programs are now available. The first permits reconstruction of neuron positions (e.g., following retrograde labeling with horseradish peroxidase) in serial sections of spinal cord, as needed for studies of motor nucleus anatomy after retrograde HRP labeling. The second set of programs allows reconstruction of the dendritic tree of an individual, intracellularly-labeled neuron directly from serial sections. The computer maintains an expanding data file containing the name of each dendritic segment, using a schema developed in LNLC by which the segment name specifies the location of any dendritic segment within the binary branching tree, plus the positions of the start and end point of the segment in three-dimensional coordinates, plus the length and diameter of each segment. Reconstruction of motoneurons which required weeks of laborious photography, hand mapping, and micrometer measurements, can now be accomplished in a relatively few hours.

The "map pin" electrode design developed in LNLC is currently being evaluated for application by a team of neuroscientists and neurosurgeons at the VA Hospital in Syracuse, New York, in neurophysiological studies in human patients undergoing craniotomy. The interest in the electrode grew out of work with the extramural Neural Prosthesis Contract Program. A collaborative project with the Surgical Neurology Branch, NINCDS, is being planned to evaluate this method for selective stimulation of the visual cortex, which is necessary to the eventual development of a practical visual prosthesis for blind patients.

A variety of other devices and techniques has been explored in this project during FY 1985, including linear electrode arrays for mapping of individual muscle unit fiber distribution in complex muscles, a laser diffraction system for studying average sarcomere length in cat muscles in situ, a "floating" microelectrode system for recording individual neurons within the spinal cord during treadmill locomotion in moving animals, and several refinements of software data processing and display systems that greatly facilitate analysis of the multichannel data streams that result from implanted transducer experiments. These and other developments are described in more detail in the project report.

All experimental work and data analysis in the project "Conduction Properties of Peripheral Nerve" was completed during FY 1985. Full reports of these results are being prepared and the project was terminated.

CONTRACT NARRATIVE
Laboratory of Neural Control, IRP, NINCDS
Fiscal Year 1985

University of Maryland: (NO1-NS-3-2348)

Title: Kinesiological Modeling of the Cat Hindlimb Musculature During Locomotion

Contractor's Project Director: Dr. William Levine, Professor of Electrical Engineering

NIH Project Officer: Dr. Gerald E. Loeb, LNLC, IRP, NINCDS

Current Funding: $59,737.00 per annum

Objectives: This is a research contract to develop a mathematical model of the biomechanics of the cat hindlimb, to implement this model as a set of computer programs, and to incorporate such data as can be provided by the LNLC regarding the structure and function of this limb as pertains to locomotion.

Major Findings: The model now consists of a five segment description of the structure and motion of the cat hindlimb in the parasagittal plane. This model has been used successfully to generate complete descriptions of the motion, accelerations, and net joint torques attributable to muscle action at each of the four included joints. This has revealed some surprising and important features of locomotion that were not intuitively obvious from traditional studies of kinesiology and electromyography (detailed in Project Z01 NS 02079-12 LNLC). It is now possible to determine the length and velocity of excursions of all 33 individual hindlimb muscles, including differences attributable to distributed origins and insertions and the relative contributions caused by independent motion at each of the joints crossed by multiarticular muscles. These are being correlated with architectural and functional features of these muscles as determined in Project Z01 NS 02080-12 LNLC. Scaling techniques have been devised to permit data drawn across many individual animals to be compared in dimensionless terms and to fill in the likely features of structure and function that surround any particular individual experiment on a restricted feature of hindlimb function. A user-friendly shell is being constructed to permit individual investigators to access and manipulate the various programs and data bases constituting the model, both to process kinesiological data and to conduct "thought-experiments" regarding motor control strategies.

Significance to the NINCDS Program and Biomedical Research: This contract provides the LNLC with access to an experienced and innovative group of engineers and applied mathematicians whose analytical techniques and technical expertise are already leading to significant improvements in experimental designs in kinesiology. It is becoming increasingly clear that intuitive notions about muscular action based on classical descriptions of anatomy and anecdotal observations of EMG are frequently misleading, or even incorrect. It is only through complete and rigorous analysis of such complexities as multiarticularity, kinematic effects on force output, and dynamic effects of inertia that we can properly appreciate the actual biomechanical function of individual muscles. Only then can we ask intelligent questions about their motor control by central programs and sensory feedback loops. It is already apparent that the control of many muscles will be found to embody control schemes that have not even been proposed, which may account for the recent discoveries of interneuronal circuits whose connectivity appears to be far more complex than was believed to be necessary. A better understanding of normal motor control will undoubtedly lead to advances in the understanding of pathological states and in their treatment of prosthetic techniques such as Functional Neuromuscular Stimulation.

Proposed Course: The contract will be continued for the remainder of its three budgeted years.

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01686-17 LNLC

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Motor Control Systems in the Spinal Cord

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R.E. Burke, M.D.	Chief	LNLC NINCDS
Other:	J.W. Fleshman, Ph.D.	Staff Fellow	LNLC NINCDS
	Idan Segev, Ph.D.	Visiting Fellow	LNLC NINCDS
	Donald E. R. Meyer, Ph.D.	Visiting Fellow	LNLC NINCDS
	Brian J. Schmidt, M.D.	Guest Researcher	LNLC NINCDS
	Diane Omeniuk, M.S.	Guest Researcher	G.W. U.
	Pablo Rudomin, Ph.D.	Fogarty Scholar-in-residence	

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neural Control

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.4	3.7	.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is designed to provide information on the mechanisms operating within reflex systems in the adult cat spinal cord, which include alpha motoneurons as the output link, as well as on the interconnections and interactions between reflex pathways and control systems descending to the spinal cord from supraspinal centers. Particular consideration is also given to interrelations between synaptic organization, intrinsic neuronal properties, and dynamic behavior of the alpha motoneurons, and the motor unit type, as defined by the physiological characteristics of the innervated muscle fibers. A variety of preparations have been used, including anesthetized, decerebrate animals as well as intact, freely moving cats. Electrophysiological and morphological data are obtained.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	**PROJECT NUMBER** Z01 NS 01687-17 LNLC

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Techniques for Making Connections with the Nervous and Musculoskeletal Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.J. Bak	Electronics Engineer	LNLC NINCDS
Other:		
R.E. Burke, M.D., Chief, LNLC	G.E. Loeb, M.D., Med Off (Res.)	LNLC NINCDS
M.C. Carter, Ph.D., Staff Fel	A.J. Rindos, Guest Researcher	LNLC NINCDS
C.M. Chanaud, Guest Researcher	E.M. Schmidt, Ph.D., Biol Eng	LNLC NINCDS
G.M. Dold, Engineering Tech	W.J. Yee, Biol Eng	LNLC NINCDS
S.H. Duenas-Jimenez, M.D. Visiting Fellow		LNLC NINCDS

COOPERATING UNITS *(if any)*
Fundamental Neurosciences Program, NINCDS (F.T. Hambrecht)

LAB/BRANCH
Laboratory of Neural Control

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	.2	1.6

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is intended to develop techniques and instrumentation for the acquisition and processing of neuroelectric signals from the central and peripheral nervous system in acute and chronic neurophysiological preparations. Because of this laboratory's continuing interest in sensorimotor neural activity during unrestrained movements, the project also includes development and fabrication of chronically implantable mechanical transducers, catheters, and connectors. Also included is the development of computer programs of general utility for acquisition and analysis of neuroelectric and mechanical records, as well as of neuroanatomical material.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01688-17 LNLC

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cortical Mechanisms of Voluntary Motor Control

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.M. Schmidt, Ph.D.	Biological Engineer	LNLC NINCDS
Other:	M.J. Bak	Electronics Engineer	LNLC NINCDS
	G.M. Dold	Engineering Technician	LNLC NINCDS
	J.S. McIntosh	Physiologist	LNLC NINCDS

COOPERATING UNITS *(if any)*

Fundamental Neurosciences Program, NINCDS (F.T. Hambrecht); Neuroprosthesis Research Program, NINCDS

LAB/BRANCH

Laboratory of Neural Control

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.9	.9	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is designed to investigate the size and spatial distribution of cortical neuron "colonies" in the primate motor cortex that project to the spinal cord and are associated with individual muscles or closely related groups of muscles, as well as the activity of neurons in such colonies during defined voluntary motor behaviors. Intracortical microstimulation (ICMS) is used to map regions that produce excitation or inhibition of particular muscles or muscle groups, and the resultant cortical maps are compared with those for synergist or antagonist muscle groups. Cortical cell discharge patterns during normal movements are evaluated with respect to the excitation or inhibition of muscle activity that is produced by ICMS. Spinal cord organization of motoneurons innervating the two heads of flexor carpi ulnaris (FCU) was explored with nerve injections of HRP. No difference was found in the size or localization of the motoneuron innervating the two heads of FCU even though the heads are composed of predominately different fiber types.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02079-12 LNLC

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Models of Neurophysiological Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W.B. Marks, Ph.D.	Research Physiologist	LNLC NINCDS
Other:	G.E. Loeb, M.D.	Medical Officer (Res.)	LNLC NINCDS
	M.M. Manley	Bio. Lab. Tech.	LNLC NINCDS
	M.C. Carter, Ph.D.	Staff Fellow	LNLC NINCDS

COOPERATING UNITS *(if any)*
Dept. of Electrical Engineering, U. MD (W.S. Levine, A.J. Rindos, Jiping He, W.M. Roberts)

LAB/BRANCH
Laboratory of Neural Control

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.3	2.5	.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

As quantitative data from a wide variety of techniques and levels of investigation become available for a particular nervous system function, it is both possible and advisable to attempt to assimilate such information into a comprehensive model of the underlying mechanisms and their interactions. This project consists of the development of such models and the necessary analytical and mathematical techniques for their implementation and testing in several areas of intensive experimental investigation by LNLC members and the scientific community at large.

The kinematic model of the cat hindlimb has predicted joint torques which suggest the function of some of the muscles of the hindlimb during locomotion. Improved analysis techniques for muscle and nerve response to stimulation during locomotion have suggested that the spinal stepping generator depolarizes terminals of muscle afferents during locomotion with a muscle-specific time course. It appears that the tensor notation for parallel processing of sensory and motor signals may be an appropriate language for modelling patterned muscle control systems.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02080-12 LNLC

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neuromuscular Coordination of Movement

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G.E. Loeb, M.D.	Medical Officer, Res.	LNLC NINCDS
Others:	W.B. Marks, Ph.D.	Research Physiologist	LNLC NINCDS
	C.A. Pratt, Ph.D.	Staff Fellow	LNLC NINCDS
	S.H. Duenas-Jimenez, M.D.	Visiting Fellow	LNLC NINCDS
	C.A. Chanaud	Guest Researcher	LNLC NINCDS
	A.J. Rindos	Guest Researcher	LNLC NINCDS
	S.A. Spector, Ph.D.	Guest Researcher	LNLC NINCDS

COOPERATING UNITS *(if any)*

Queen's University Hospital, Dept. of Physiology, Canada (F.J. Richmond)

LAB/BRANCH

Laboratory of Neural Control

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS: 4.4	**PROFESSIONAL:** 3.4	**OTHER:** 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The cat has long been a standard animal for anatomical and acute physiological studies of muscle function and motor control at the spinal cord level. In this project, a wide variety of traditional and novel kinesiological techniques are being used to study motor tasks in unanesthetized, normally behaving cats, including computer-aided reconstruction of skeletal movement from videotape, multiaxis force plates, chronically implanted nerve cuff and EMG electrodes, and strain and length transducers. The major focus has been the study of hindlimb muscles and their afferent and efferent control during walking, which is the subject of a computer modeling project described in Project No. Z01-NS-02079-12 LNLC. Other hindlimb movements studied include jumping, paw shaking, scratching, and reflexes to cutaneous nerve stimulation during normal and decerebrate walking. In a collaborative study, similar data are being collected from a large number of neck muscles.

The major objective is to correlate patterns of usage with the complex mechanics and compartmentalization and proprioceptive specializations of these muscles. A major theme emerging from these experiments is a concept of "Task Groups," which denotes the segregation and specialization of sensorimotor systems to perform kinematically homogeneous tasks in an optimal manner. This is particularly apparent in multiarticular muscles, which in some cases use independent subdivisions of their alpha motoneuron pool to accomplish kinematically diverse tasks. Some of these bifunctional muscles have been found to have a heretofore overlooked internal architecture consisting of short, parallel muscle fibers in series, which poses additional questions regarding their coordination.

Current work asks how well these notions extend to other bifunctional muscles and other programs (such as reflexes) and is examining how much anatomical and physiological independence exists between task groups, in both the spinal cord and in the muscle.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02160-11 LNLC

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Intrinsic Properties of Motor Units

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.E. Burke, M.D.	Chief, LNLC	LNLC NINCDS
Others:	J.W. Fleshman, Ph.D.	Staff Fellow	LNLC NINCDS
	C.A. Pratt, Ph.D.	Staff Fellow	LNLC NINCDS
	I. Segev, Ph.D.	Visiting Fellow	LNLC NINCDS

COOPERATING UNITS *(if any)*
Mathematics Research Branch, NIADDK (W. Rall); Dept. of Anatomy, Hadassah Medical School, Jerusalem, Israel (A. Lev Tov)

LAB/BRANCH
Laboratory of Neural Control

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	1.4	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is designed to provide information on the ranges and distributions of the electrophysiological and morphological characteristics of alpha motoneurons and of the interrelated mechanical, histochemical and morphological properties of the muscle fibers innervated by them (i.e., the muscle unit) in various hindlimb muscles in the cat. Methods used include intracellular recording and stimulation, measurement of mechanical properties of muscles and individual muscle units, neuroanatomical techniques of intracellular staining with horseradish peroxidase, along with conventional and computer-aided methods for reconstruction of extensive neuronal structures from serial histological sections, and computer modeling and data processing. In some experiments, motor unit populations in normal animals are compared with those in animals after various conditioning treatments. Studies of alpha motoneuron properties are included in this project when they are related importantly to the type of muscle unit innervated by the studied cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	**PROJECT NUMBER** Z01 NS 02534-03 LNLC

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Conduction Properties of Peripheral Nerve

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G.E. Loeb, M.D.	Medical Officer (Res)	LNLC NINCDS
Other:	A.J. Rindos	Guest Researcher	LNLC NINCDS

COOPERATING UNITS *(if any)*
Neuroimmunology Branch, NINCDS (C. Krarup)

LAB/BRANCH
Laboratory of Neural Control

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.4	.3	.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is concerned with the electrical conduction properties of normal and damaged peripheral nerves. Nerve cuff electrodes with multiple recording and stimulation contacts have been chronically implanted on the sciatic and posterior tibial nerves of the cat hindlimb for periods of up to one year. Using various electrophysiological and histological techniques, it has been demonstrated that such devices can provide quantitative measurements of the numbers of fibers conducting at various velocities without the devices themselves causing any significant changes in the nerves. These devices and techniques have been used to study the effects of crush and constricting lesions of peripheral nerves on their conduction and to provide a quantitative, longitudinal study of regeneration distal to such lesions.

The experimental studies have been completed and work under this project is now related exclusively to completing the data analysis and preparation of journal articles describing the findings and the novel techniques.

TAB 8 -- LABORATORY OF NEUROBIOLOGY -- (LN)

ANNUAL REPORT
October 1, 1984 through September 30, 1985
Laboratory of Neurobiology
National Institute of Neurological
and Communicative Disorders and Stroke

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ANNUAL REPORT
October 1, 1984 through September 30, 1985
Laboratory of Neurobiology, IRP
National Institute of Neurological and Communicative
Disorders and Stroke

Thomas S. Reese, Chief

The Laboratory of Neurobiology has two Sections, the Section on Structural Cell Biology and the Section on Structural Plasticity. The Section on Structural Cell Biology uses modern structural and biochemical techniques to investigate basic cell biological problems germane to an understanding of the function of nerve cells; the Section on Structural Plasticity applies these and other appropriate approaches directly to problems of both fundamental and clinical importance in the mammalian central nervous system, emphasizing problems related to regeneration and response to injury. Current emphasis of the the Section on Structural Cell Biology is on the mechanism of axoplasmic transport and axonal growth while the Section on Structural Plasticity is investigating factors which promote establishment of connections and blood-brain-barrier funtion in neural tissues implanted in the brain.

Considerable progress has been made in the Section on Structural Cell Biology in understanding the directed organelle movements which move materials by fast axoplasmic transport. Filaments can be isolated from the axoplasm of the squid giant axon which support directed movements of organelles for many hours, at 1-2 um per sec, provided adenosine triphosphate (ATP) is present. These organelles and filaments are below the resolution limit of the light microscope so fast digital image processing of differential interference contrast images is required to visualize them. Subsequent direct freezing and metal replication of filaments previously observed with the light microscope provide a means to show with the electron microscope that their central structure is a single microtubule and that the various organelles moving along them are closely attached. Because organelles of all sizes, including mitochondria, move along these filaments at the same rate, it seems likely that all the organelle movements of fast axoplasmic transport are powered by a single "molecular motor". Differences in the rates of transport in intact axoplasm are now thought to be determined by impeding interactions of organelles with other axoplasmic components.

Progress has been made towards characterizing the translocator for axonal transport. Treatment of latex beads with a crude extract of squid brain or axon induces the beads, in the presence of ATP, to move along microtubules reconstituted from pure tubulin. Thus the neuron appears to contain a free pool of translocator which binds to a substrate and exerts directed forces on microtubules. Affinity for microtubules in AMP-PNP (a nonhydrolysable analogue of ATP) has been used to purify from squid brain a 700 KD protein with 110 KD and 60-65 KD doublet peptides which, with ATP, attach to a substrate and induce it to move in one direction along a microtubule. A monoclonal antibody column (directed towards the 110 KD subunit) was then used to purify this translocator from squid brain. Active translocator which eluted off the antibody column at high ph had the same component subunits in the same stochiometry as the translocator purified by its microtubule affinity in AMP-PNP. Thus the active form of this protein appears to be a polypeptide complex.

Based on its size and pharmacological properties this protein is neither a dynein nor a myosin, so we have defined a new class of motility proteins which we call kinesin. Kinesin occurs outside of neural cells and may be of general significance in cellular motility. However, organelle movement induced by kinesin has only one direction, away from the cell body as determined by observing kinesin-induced movement of latex beads along microtubules made from centrosomes, which have a defined polarity. Therefore kinesin could only mediate anterograde axonal transport. Indeed, the flow-through from the antibody column, which is stripped of its kinesin, induces bead movement in the retrograde direction. Our current efforts are concentrated on purifying this retrograde translocator.

In order to develop further a realistic picture of the detailed organization of cytoplasm, monolayers of cultured myocytes and neurons were directly frozen and examined in a 200 kV electron microscope to determine the structure of the cytoplasmic "ground substance" lying between the major filamentous elements and to determine how organelles move through these filamentous elements. This approach has also provided a more detailed understanding of the organization of cytoplasm. A matrix of fine (ca 4 nm) filaments links the major filamentous elements; the soluble proteins and other granular components of cytoplasm are embedded in this fine filament meshwork. Their density and architecture differs in different regions of the cell, and are related to the characteristics of organelle movements in these different regions. Organelles observed moving along microtubule tracks make cross-bridges with microtubules and assume a streamlined, tear-drop shape suggesting that the moving organelle is subjected to viscous forces when it is pulled through the cytoplasmic matrix.

Axon terminals on lizard intercostal muscles are unique in lying close enough to the surface of the muscle to be rapid frozen, freeze substituted, and stained with block stains permitting a three-dimensional reconstruction of their cytoplasmic structures. These new freeze-substitution techniques have shown that neurofilament bundles in the axon are continuous, but in the axon terminal they are interrupted by discrete structures (discontinuity plaques) which contain various membrane-limited organelles. These plaques are likely sites for neurofilament degradation since the filaments are thought to be transported down the axon and degraded by proteases in the terminal. How proteases, synaptic activity, and extracellular calcium affect the turnover of neurofilaments in the presynaptic terminal is now under investigation.

Direct freezing and improved freeze-substitution techniques have been applied to growing tips of neuronal processes during development of synaptic connections in the chick optic tectum. Numerous flattened vesicles are found in groups near the growth cone surface; their total area approaches that of the plasmalemma. These membranes would be available to support the rapid expansion of the growth cone surface. Experiments are underway which show that these membranes can join the surface membrane at the tip of the growth cone, and that material internalized from the surface reaches the vicinity of these vesicles. Therefore, a system for recycling membrane through the plasmalemma of the elongating axonal tip is being defined by these studies.

Freeze fracture views of adult synapses on lizard and frog muscle showed that structural differences in the membrane organization of neurotransmitter release sites are correlated with physiological differences in the quantal release of transmitter, depending on whether the synapse is with a twitch (fast) or tonic (slow) muscle fiber. These structural differences support our earlier hypothesis that the large intramembrane particles found at transmitter release sites are the calcium channels responsible for depolarization-dependent transmitter release because their organization in the presynaptic membrane provides a clear explanation of how levels of quantal transmitter output are determined at different types of synapses. We are currently comparing high with low output synapses in invertebrates to see whether the organization of their transmitter release sites supports this hypothesis.

Methods are now well established for preparing, from rapidly-frozen tissues, thin cryosections in which dislocations of soluble, diffusible elements are negligible. Thus, quantitative compositional analysis is now routine, and structural imaging of cryosections is becoming quite acceptable, despite the poor sectioning properties and inherently low contrast of unfixed, unstained, and well-frozen tissues. These techniques have been used to study quantitatively the distribution of calcium at synapses and to determine the relationship of this distribution to neuronal activity. In both resting cholinergic synaptosomes and in resting parallel fiber to Purkinje cell synapses of the mouse cerebellar cortex, elemental imaging has demonstrated the absence of calcium "stores", that is, intracellular compartments which might release enough calcium upon depolarization to produce calcium "second messenger" effects. The cerebellar cortex does, however, have postsynaptic sites, possibly, the smooth-membrane cisterns of Purkinje cell spines, that accumulate calcium derived from extracellular sources in response to synaptic depolarization. These findings have significant implications, not only for the role of calcium in neurotransmission, but also for calcium involvement in the formation and maintenance of synaptic contacts. Thus, it has become important to determine if calcium-sensitive assemblies of structural proteins might be regulating characteristic structural features of these synapses, e.g., dendritic spines. For these purposes, immunocytochemical methods for localizing cytoskeletal proteins in frozen sections (0.5 um thick or less) have been developed. Preliminary applications, revealing the abundance of actin and spectrin at sites of active myelination, demonstrate that this method has the resolution, sensitivity and accessibility for studies of small neuronal compartments. Another new approach under development is the application of antimony-based analogs of acetylcholine (ACh), which are known to be biochemically similar to ACh and which can be detected in the analytical electron microscope, to determine the sites of ACh storage and release.

The Section on Structural Plasticity has recently been concerned with the possibility of reconstructing a neuroendocrine circuit in an accessible portion of the cerebrospinal fluid (CSF) compartment, the IV ventricle. The CSF, which communicates with the extracellular fluid of the brain, may thus mediate interactions between brain and grafts placed within it. Fragments of superior cervical ganglion (SCG), allografted to the IV ventricle, become rapidly vascularized and survive indefinitely. The next step was to co-graft one of the SCG's targets, the pineal gland, to pinealectomized recipients. The goal was to see whether a disrupted neuroendocrine circuit, retina-hypothalamus-spinal cord-SCG-pineal gland, could be reconstructed upon the surface of an otherwise normal brain. An integral part of the attempt was to learn whether the grafts not only survived, but were able to perform their function, the secretion of melatonin. To this end, urinary 6-hydroxymelatonin (6-HO-M) was measured over a 24-hour period. Pineal allografts persisted and retained much of their normal architecture. The identification of their parenchymal cells as pinealocytes was established immunohistochemically and ultrastructurally. However, a single pineal allograft produced no detectable melatonin. It was not until 5 to 8 pineal glands had been transplanted, that appreciable amounts of 6-HO-M were recovered in the urine. The SCG implant sent bundles of unmyelinated axons to pinealocytes and capillaries within the adjacent pineal grafts. Pineal allografts become innervated by SCG co-transplants but a sufficient volume of pineal tissue must be inserted into the IV ventricle in order to yield appreciable amounts of secretory product.

In assessing the roles of extracellular matrix and of target tissue in the regeneration of axons within the central nervous system (CNS), an acellular conduit: a stainless steel cannula, was inserted into the corpus callosum of adult rats. The lumen of the tube was occluded by an obturator so as to prevent herniation of brain tissue into the tube during insertion. The exterior, free end of the placement of the cannula, the obturator was removed. At six weeks, regenerating callosal axons had entered the cannula. By 16 weeks, the regenerating core of tissue consisted of densely packed fascicles of unmyelinated axons, myelinated and myelinating axons, a few growth cones, many glial cells, degenerating myelin, and capillaries. All of these elements formed strikingly parallel columns that extended toward the dorsal surface of the brain, at about 90° from their normal, transverse course. The elongation of axons did not exceed about 1.3mm. Thus, mature axons of the CNS can, without benefit of a pre-existing substrate, regenerate into an acellular tube for a limited distance in the absence of target tissue. The ingrowing neuronal, glial and endothelial processes are, apparently, able to produce their own substrates which are conducive to a restricted axonal growth and remyelination.

The morphological reactions to focal injury of the brain's surface, a related problem, involves rapidly developing intramembrane changes in two cell types and a slower alteration in the cytoplasm of one of them. The increase in the number of intramembrane particle assemblies in astrocytes, examined after freeze-fracturing, is accompanied by an equally rapid development of tight and gap junctions within the plasma membranes of adjacent arachnoid cells. These events take place from 30 minutes to 3 hours following injury. A slower change, requiring about 24 hours, is the first appearance, detected immunohistochemically, of glial fibrillary acidic protein (GFAP) within astrocyte cytoplasm. Since the increase in the assemblies precedes the appearance of GFAP, it is unlikely that glial intermediate filaments, the source of GFAP antigen, are directly involved in the insertion of new assemblies into the cell membrane. The remarkably extensive development of tight junctions between reactive arachnoid cells indicates that a damaged arachnoid membrane is quickly resealed.

Although, in vitro, cerebral endothelial cells can form tight junctions (TJ) and astrocytes can make and intercalate assemblies into their cell membrane, both structures are relatively few. By co-culturing these cells, we have found a structural interaction. When endothelial cells from beef brain, or their conditioned medium, are added to astrocyte cultures, some endothelial cells are joined by TJ that are about twice as extensive (average length of 5.4u) as are those in endothelial cells grown alone (average length of 2.8u). In co-cultures, 5 of the TJ measured were longer than 10u, the longest being 19.5u. The longest TJ in controls was no greater than 4.3u, to date. The TJ in co-cultures were also more complex: there were more strands and connections between them. Some astrocytes receiving medium conditioned by endothelial cells contained 5 to 10 times more assemblies than astrocytes maintained alone. Some of the assemblies were also clustered rather than randomly distributed. Thus, the addition of astrocytes results in the formation of TJ more nearly resembling in situ barrier junctions in their extent and complexity. Conversely, the addition of endothelium to astrocytes makes the number of assemblies more comparable to that of perivascular astrocytes in situ. However, the modulation of TJ and assemblies may be completely independent of one another.

The conditions for maximal penetration of blood-borne proteins into the brain, from non-neural grafts to the brain, have been examined. Autografts of skeletal (nuchal) muscle and skin were placed on the pial surface (S) of medulla and cerebellum or into the parenchyma (P) of adult rats. S grafts consistently became innervated by, presumably, collateral sprouts from cranial nerves and survived for at least 1 year. P grafts did not become innervated and survived for no more than 6 months. Endogenous IgG (MW 160,000), detected immunohistochemically, entered the brain around the grafts for a very limited distance compared with blood-borne horseradish peroxidase (HRP) (MW 40,000). The furthest penetration into the brain of HRP was greater from S grafts (4.6mm) than from P grafts (2.2mm). S grafts that were only 0.5mm longer and wider than other grafts permitted significantly greater penetration (1.6 vs 0.9mm). Thus, large, pial grafts are the most effective ones to bring blood-borne macro-molecules, normally exculded by the blood-brain barrier (BBB), into the brain.

Receptor mediated endocytosis, in contrast to bulk phase endocytosis, is being studied to learn how cerebral endothelium may selctively transport large molecules across the blood vessel wall. Like endothelium from other organs, brain endothelial cells in vitro avidly endocytose fluorochrome-tagged, acetylated, low density lipoprotein (AcLDL). This "scavenger," bulk uptake results in a pronounced labeling, seen with fluorescence microscopy, of endothelial lysosomes at 3 to 4 hours, via numerous, endocytosing pits and vacuoles, visualized by electron microscopy, with wheat germ agglutinin (WGA) lectin-HRP. A few large, coated pits and adjacent segments of cell membrane, some small vesicles, and a few tubules become heavily labeled with peanut and asparagus lectins. The sugar residues thus labeled are much more restricted on the cell surface than those bound to WGA lectin and may be related to receptor mediated endocytosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01442-19 LN

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Permeability of cellular layers in the vertebrate nervous system

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

T.S. Reese, Chief, Laboratory of Neurobiology, NINCDS
B. Kachar, Visiting Associate, Laboratory of Neurobiology, NINCDS

COOPERATING UNITS (if any)

Marine Biological Laboratory, Woods Hole, MA 02543
R.P. Rand, Brock University, St. Catherine's, Ontario, Canada
J.S. Handler, KE, IR, NHLBI, NIH, Bethesda, MD

LAB/BRANCH

Laboratory of Neurobiology

SECTION

Section on Structural Cell Biology
(Located at the Marine Biological Laboratory, Woods Hole, MA 02543)

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	0.8	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The substructure of tight junctions is investigated by direct freezing techniques that avoid any chemical fixation and serve to increase the resolution of individual membrane components. The backbone of the tight junction of each of the paired component membranes is a continuous cylinder. This model replaces the previous view that tight junctions are comprised of rows of intramembrane proteins; the rod-shaped structures are now interpreted as inverted cylindrical micelles of membrane lipids. Evidence for this model is also being gathered from investigations of pure lipid bilayer systems which are induced to form non-planar micellar phases by addition of calcium ion. Cylindrical micelles identical to those seen at tight junctions are found embedded in these lipid bilayers. Tight junctions, but not septate junctions, in invertebrates appear to have lipidic backbones. How tight junctions serve in the blood-brain barrier system to prevent small charged solutes from entering the brain is made clear by this new model of tight junction structure. Gap junctions form within minutes of incubation of prostate slices in certain media, even when it contains metabolic inhibitors. This result suggests that precursors of the intramembranous components of gap junctions preexist in the cell membrane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01881-15 LN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Structural basis of synaptic transmission

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

T.S. Reese, Chief, Laboratory of Neurobiolcgy, NINCDS
J. Walrond, Staff Fellow, LN, NINCDS
T. Cheng, Visiting Fellow, LN, NINCDS
B. Kachar, Visting Associate, Laboratory of Neurobiology, NINCDS

COOPERATING UNITS (if any)
Marine Biological Laboratory, Woods Hole, MA 02543
D. Landis, Dept. of Neurology, Massachusetts General Hospital, Boston, MA
R.A. Altschuler and J. Fex, LNO, NINCDS, NIH, Bethesda, MD

LAB/BRANCH
Laboratory of Neurobiology

SECTION
Section on Structural Cell Biology
(Located at the Marine Biological Laboratory, Woods Hole, MA 02543)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.2	2.0	1.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

New areas of investigation of synaptic structure are underway. A method for staining freeze-substituted tissue has been developed which requires no further stain after the sections are cut, so the stain extends evenly through the section. Therefore the three dimensional structure of the cytoskeleton and related fine filaments in synapses can be determined in continuous serial sections. How neurofilaments end in synaptic terminals has been determined; this is important because neurofilament lengths are thought to be regulated by Ca-activated proteases at their terminations. Application of the freeze-fracture techniques has shown that the pattern of active zone structure at synapses on fast muscle fibers differs from that on slow muscle fibers; these structural differences provide a basis for understanding why terminals or fast fibers release more transmitter quanta than those on slow fibers. Growing nerve terminals in the brain have been reconstructed from serial sec- tioned freeze-substituted preparations. These new preparative methods have revealed an internal system of membranes which are thought to be the source of the new membrane added to the surface of the growth cone during its growth. These membranes are highly labile and are destroyed by conventional fixatives. Current evidence indicates that they participate in recycling of membranes needed for extension of the growth cone.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02551-04 LN

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure of neuronal cytoplasm

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

T.S. Reese, Chief, Laboratory of Neurobiology, NINCDS
B.J. Schnapp, Staff Fellow, Laboratory of Neurobiology, NINCDS
B. Kachar, Visiting Associate, Laboratory of Neurobiology, NINCDS
V. Aviv, Guest Worker, Laboratory of Neurobiology, NINCDS

COOPERATING UNITS *(if any)*

Marine Biological Laboratory, Woods Hole, MA 02543
M. Sheetz, Univ. of Connecticut Health Center, Farmington, CT
R. Vale, Stanford Medical School, Stanford, CA

LAB/BRANCH

Laboratory of Neurobiology

SECTION

Section on Structural Cell Biology
(Located at the Marine Biological Laboratory, Woods Hole, MA 02543)

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.5	4.5	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project determines the structure of neuronal and glial cytoplasm, particularly as it pertains to axoplasmic transport, and the organization of the cytoplasm. Cultured myocytes, grown on grids, frozen, freeze-substituted, and examined directly at high voltages in an electronmicroscope have a cytoplasmic ground substance consisting of fine filaments instead of a microtubular meshwork, and distinct cytoplasmic domains characterized by different types of organelle movements. Filaments are isolated from the axoplasm of the squid giant axon along which organelles continue to move for many hours. Filaments previously observed with the video microscope and then examined in the electronmicroscope turn out to be single microtubules. However, similar filaments in certain cells are made of actin, so organelle movements may be actin based in some locations and microtubule based in others. Progress has been made towards characterizing the translocators for these organelle movements. Treatment of latex beads with a crude extract of squid brain induces the beads to move along microtubules. A 700 KD protein with 110 KD and 60-65 KD doublet peptides which was then purified moves beads along microtubules. A monoclonal antibody column (directed towards the 110 Kd subunit) was also used to purify this translocator Based on its size and pharmacological properties this translocation is neither a dynein nor a myosin, so we have defined a new class of motility protein which we call kinesin. Kinesin appears to be of general significance in cellular motility. However, organelle movement induced by kinesin only translocates in a direction corresponding to anterograde axonal transport. However, brain extracts stripped of kinesin with antibody induce bead movement in the retrograde direction. Our current efforts are concentrated on purifying the retrograde translocator and determining whether it is selectively bound to some organelles.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02610-02 LN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Distribution of Mobile and Structural Components at Chemical Synapses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

S.B. Andrews, Special Expert, Laboratory of Neurobiology, NINCDS
T.S. Reese, Chief, Laboratory of Neurobiology, NINCDS

COOPERATING UNITS *(if any)* Roger C. Wagner, University of Delaware, Newark, DE 19711
Charles E. Fiori and Richard D. Leapman, BEIB, DRS, NIH, Bethesda, MD
Bruce D. Trapp, Johns Hopkins University School of Medicine, Baltimore, MD
Mordecai P. Blaustein, University of Maryland Medical School, Baltimore, MD

LAB/BRANCH
Laboratory of Neurobiology

SECTION
Section on Structural Cell Biology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	1.4	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project aims to determine the distribution of diffusible and structural components at chemical synapses. This work is significant because of the relationship between the localization and movement of such constituents and their role in synaptic transmission. To attain the resolution and sensitivity necessary to study synapses, this project combines several significant technological advances, including rapid freezing and cryosectioning of unfixed tissues, cryosectioning and immunocytochemical staining of sucrose-protected tissues, and quantitative, element-specific x-ray imaging and analysis in a specialized analytical electron microscope. Studies of the intracellular calcium distribution in the molecular layer of mouse cerebellum indicate that presynaptic calcium stores are not present and are not required for the activity of parallel fiber/Purkinje cell synapses. Membrane depolarization, however, is accompanied by the loading of extracellular calcium into organelles in the dendrites of Purkinje cells, demonstrating a requirement for calcium-handling organelles in postsynaptic elements. Analysis of calcium distribution in the synaptosomes from squid brain confirm the absence of presynaptic calcium stores in resting cholinergic terminals. This preparation is also being used, in conjunction with an antimony-labeled acetylcholine analog, to determine where ACh is taken up and stored in cholinergic synaptosomes. Correlative studies on structural aspects of synaptic function have shown that rapid freezing is essential for preserving the native organization of labile membrane structures such as vesicles. Immunocytochemical studies have supported the involvement of actin and brain spectrin in myelination, and provided an approach to determining the role of cytoskeletal proteins in the organization of brain synapses. Thus, this project has now begun to provide important information on the detailed relationship between the diffusible and structural components of synapses, and how these regulate synaptic activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 01805-17 LN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Membrane Structure of Astrocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

S. Cheng, Staff Fellow, LN, NINCDS
Z. Nagy, Visiting Associate, LN, NINCDS
J. Anders, Guest Worker, LN, NINCDS
M. Brightman, Head, Section on Structural Plasticity, LN, NINCDS

COOPERATING UNITS (if any)
Laboratory of Chemical Pharmacology, NCI

LAB/BRANCH
Laboratory of Neurobiology

SECTION
Section on Structural Plasticity

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS: 2.4	PROFESSIONAL: 2.3	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although, in vitro, cerebral endothelial cells can form tight junctions (TJ) and astrocytes can make and intercalate assemblies into their cell membrane, both structures are relatively few. By co-culturing these cells, we have found a structural interaction. When endothelial cells from beef brain, or their conditioned medium, are added to astrocyte cultures, some endothelial cells are joined by TJ that are about twice as extensive (average length of 5.4u) as are those in endothelial cells grown alone (average length of 2.8u). In co-cultures, 5 of the TJ measured were longer than 10u, the longest being 19.5u. The longest TJ in controls was no greater than 4.3u, to date. The TJ in co-cultures were also more complex: there were more strands and connections between them. Some astrocytes receiving medium conditioned by endothelial cells contained 5 to 10 times more assemblies than astrocytes maintained alone. Some of the assemblies were also clustered rather than randomly distributed. Thus, the addition of astrocytes results in the formation of TJ more nearly resembling in situ barrier junctions in their extent and complexity. Conversely, the addition of endothelium to astrocytes makes the number of assemblies more comparable to that of perivascular astrocytes in situ. However, the modulation of TJ and assemblies may be completely independent of one another.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02086-12 LN

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regeneration in Transplanted Peripheral and Central Neurons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

N.A. Azzam, Guest Worker, LN, NINCDS
M.W. Brightman, Head, Section on Structural Plasticity, LN, NINCDS

COOPERATING UNITS *(if any)*

Georgetown University, Department of Anatomy

LAB/BRANCH

Laboratory of Neurobiology

SECTION

Section on Structural Plasticity

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In assessing the roles of extracellular matrix and of target tissue in the regeneration of axons within the central nervous system (CNS), an acellular conduit: a stainless steel cannula, was inserted into the corpus callosum of adult rats. The lumen of the tube was occluded by an obturator so as to prevent herniation of brain tissue into the tube during. The exterior, free end of the tube was capped with gel foam rather than tissue. One week after stereotactic placement of the cannula, the obturator was removed. At six weeks, regenerating callosal axons had entered the cannula. By 16 weeks, the regenerating core of tissue consisted of densely packed fascicles of unmyelinated axons, myelinated and myelinating axons, a few growth cones, many glial cells, degenerating myelin, and capillaries. All of these elements formed strikingly parallel columns that extended toward the dorsal surface of the brain, at about 90° from their normal, transverse course. The elongation of axons did not exceed about 1.3mm. Thus, mature axons of the CNS can, without benefit of a pre-exisiting substrate, regenerate into an acellular tube for a limited distance in the absence of target tissue. The ingrowing neuronal, glial and endothelial processes are, apparently, able to produce their own substrates which are conducive to a restricted axonal growth and remyelination.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02144-11 LN

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Blood-Brain Barrier

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

M. Brightman, Head, Section on Structural Plasticity, LN, NINCDS
S. Wakai, Visiting Fellow, LN, NINCDS
Z. Nagy, Visiting Associate, LN, NINCDS

COOPERATING UNITS (if any)

Laboratory of Chemical Pharmacology, NCI

LAB/BRANCH

Laboratory of Neurobiology

SECTION

Section on Structural Plasticity

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.2	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
    ☐ (a1) Minors
    ☐ (a2) Interviews
☐ (b) Human tissues
☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The conditions for maximal penetration of blood-borne proteins into the brain, from non-neural grafts to the brain, have been examined. Autografts of skeletal (nuchal) muscle and skin were placed on the pial surface (S) of medulla and cerebellum or into the parenchyma (P) of adult rats. S grafts consistently became innervated by, presumably, collateral sprouts from cranial nerves and survived for at least 1 year. P grafts did not become innervated and survived for no more than 6 months. Endogenous IgG (MW 160,000), detected immunohistochemically, entered the brain around the grafts for a very limited distance compared with blood-borne horseradish peroxidase (HRP) (MW 40,000). The furthest penetration into the brain of HRP was greater from S grafts (4.6mm) than from P grafts (2.2mm). S grafts that were only 0.5mm longer and wider than other grafts permitted significantly greater penetration (1.6 vs 0.9mm). Thus, large, pial grafts are the most effective ones to bring blood-borne macromolecules, normally excluded by the blood-brain barrier (BBB), into the brain.

Receptor mediated vs bulk phase, endocytosis is being followed to learn how cerebral endothelium may selectively transport large molecules across the BBB. Like endothelium from other organs, brain endothelial cells in vitro avidly endocytose fluorochrome-tagged, acetylated, low density lipoprotein (AcLDL). This "scavenger," bulk uptake results in a pronounced labeling, seen with fluorescence microscopy, of endothelial lysosomes at 3 to 4 hours, via numerous, endocytosing pits and vacuoles, visualized by electron microscopy, with wheat germ agglutinin (WGA) lectin-HRP. A few large, coated pits and adjacent segments of cell membrane, some small vesicles, and a few tubules become heavily labeled with peanut and asparagus lectins. The sugar residues thus labeled are much more restricted on the cell surface than those bound to WGA lectin and may be related to receptor mediated endocytosis.

PHS 6040 (Rev. 1/84)

GPO 914-918

TAB 9 -- LABORATORY OF NEUROCHEMISTRY -- (LNC)

ANNUAL REPORT

October 1, 1984 through September 30, 1985

Laboratory of Neurochemistry
National Institute of Neurological and
Communicative Disorders and Stroke

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Enzymological Aspects of Neural Functions 9
Z01-NS-00813-24 LNC

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Z01-NS-02605-02 LNC

Structure and Function in Retinal Neurons 11
Z01-NS-02631-02 LNC

ANNUAL REPORT

October 1, 1984 through September 30, 1985

Laboratory of Neurochemistry
National Institute of Neurological and
Communicative Disorders and Stroke

Table of Contents

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Z01-NS-02142-11

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Z01-NS-00813-24 LNC

Mechanism of Mast Cell Secretion and Membrane Generation 10
Z01-NS-02605-02 LNC

Structure and Function in Retinal Neurons 11
Z01-NS-02631-02 LNC

ANNUAL REPORT
October 1, 1984 through September 30, 1985
Laboratory of Neurochemistry, Intramural Research
National Institute of Neurological and Communicative
Disorders and Stroke
R. Wayne Albers, Acting Chief

The Laboratory of Neurochemistry is composed of three sections: Enzyme Chemistry, Cellular Neurochemistry, and Neuronal Development and Regeneration.

Research in the Section on Enzyme Chemistry is centered on the roles of ion transport and intracellular regulation as related to neural functions. The mechanism and regulation of the Na,K-ATPase is the major project.

In a continuing study of the transient kinetics of phosphorylation of the Na,K-ATPase, we have recently established that under certain conditions both the Na^+-initiated phosphorylation and the K^+-initiated dephosphorylation of the enzyme are biphasic in Electrophorus membrane preparations. However, we have been able to prepare two different soluble forms of the enzyme, one of which retains the biphasic kinetics and one of which displays simple exponential dephosphorylation. Other observations together with these suggest that the transport-competent ATPase may require oligomeric interactions of a higher order than has been recognized previously.

A series of monoclonal antibodies are being developed as structural and functional probes of the Na,K-ATPase. In conjunction with new approaches to the solubilization and purification of the brain Na,K-ATPase, these antiodies will also be employed in the characterization of the transport system in different brain cell types.

Current studies are developing improved tchniques for the expression of Elecrophorus mRNA in in vitro translation systems and for the detction of translation products.

Another project of the Section on Enzyme Chemistry involves studies of the process of granule activation nd secretion in mast cells. Of particular interest is the high calcium content within mas cell secretory granules. Evidence has been obtained which suggests that the matrix of these granules contains preformed memrane components that may be rapidly inserted into the granule membrane during the activation process. Calcium and calmodulin have also been detected in the granule matrix and their role in the secretion process is under investigation. These sudies may provide useful insights into the control of scretion as it relates to neurotransmitter release.

Metabolic sequelae to transient brain ischemia are under investigation in the Section on Cellular Neurochemistry. Most acute alterations in metabolite levels are reversed in minutes after cerebral reperfusion. Exceptions are glycogen and protein synthesis. Because transient cerebral ischemia has long-term effects leading to selective neuronal death, these may be important clues to the proximate cause of ischemic damage to brain functions.

Current studies are focussed on the mechanism of inhibition of brain protein synthesis which follows not only transient ischemia, but also hyperthermia and electroconvulsive shock. This work has demonstrated that the suppression of brain protein synthesis is accompanied by elevated synthesis of the "heat shock" proteins. These proteins are believed to play a basic role in the regulation of gene epression in response to stress. Despite the lobal suppression of brain protein synthesis, we have found the levels of several opioid peptides to be unchanged. An apparent exception is dynorphin which was found to be depleted for as long as 24 hours following 5 minutes cerebral ischemia in gerbils.

Current and projected experimentation is designed to elucidate the molecular basis for suppression of protein synthesis. Factors under study include the possible roles of arachidonic acid release and of the phosphorylation state of protein initiation factors. A collaborative study is designd to determine the levels of ubiquitin mRNA present in post-ischemic brain. Additional experiments are planned to extend the data on the consequences of transient ischemia on levels of brain peptides.

Another project in the Section on Cellular Neurochemistry concerns the specialized metabolism of retinal neurons and, in particular, guanine nucleotide metabolism. Quantitative ultramicrochemical techniques have been applied to determine cGMP levels in the different histological layers of canine retina. The elevated levels of cGMP in whole retinas have been shown to ocur during the course of the innerited rod-cone dysplasia of the Irish setter. Quantitative ultramicroanalysis of the different retinal layers reveals that this elevation is most marked in the outer plexiform layer, increasing as much as 23 times control levels. This accumulation appears to accompany a block in the normal differentiation process of photoreceptor neurons. Current studies are obtaining corresponding data for guanylate cyclase activities in these dystrophic retinas.

The general research goals of the Section on Neuronal Development and Regeneration are to determine how grafts might be used to promote the repair of nervous tissue and how extraneuronal factors (i.e., extracellular matrix and trophic agents) influence nerve fiber regeneration.

Since foreign tissue grafts may be needed to repair nervous tissue, a series of studies were performed to find ways to overcome the destructive immune reaction elicited by the transplantation antigens present on these grafts. . It was found that the immunosuppressive agent Cyclosporin-A (Cy-A) prevented graft rejection but did not induce tolerance, since after the cessation of Cy-A treatment, the graft was rejected. Accordingly, experiments were designed to determine if the antigenic cells of the graft might be eliminated or if they would disappear after prolonged periods of transplantation. In one study, rats were perfused with a physiological salt solution to remove blood from their nerves. This manipulation did not prevent the rejection of these nerves, indicating that blood alone is not the only antigenic component of nerve. In another study, long-term surviving nerve allografts were retransplanted to non-immunosuppressed hosts. These grafts were also rejected, demonstrating that the antigenic cell type(s) persists, even after prolonged transplantation. An important new finding was that when donor blood (given intravenously) was used in conjunction with a short course of Cy-A, allogeneic neurons survived after three months whereas they were rejected in hosts given only blood or the drug. Future studies will investigate the duration and mechanism of this combined immunosuppressive protocol and whether it can be extended to other species.

In order to understand factors involved in nerve fiber regeneration, the binding of lectins to the cell surface and extracellular matrix of normal nerve was studied. This data will be correlated with binding that occurs during peripheral nerve degeneration and regeneration and comparisons made with injured central nervous system tissue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02256-09 LNC

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metabolic Profiles in Normal and Diseased Retina

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. I.	:	J. V. Passonneau	Chief, Sec. on Cellular Neurochemistry	LNC	NINCDS
Others	:	E. K. Barbehenn	Expert	LNC	NINCDS
		C. A. Gagnon	Biologist	LNC	NINCDS

COOPERATING UNITS (if any)

Laboratory of Vision Research, NEI
University of Pennsylvania School of Veterinary Medicine

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

Section on Cellular Neurochemistry

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.6	1.4	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The focus of this project is to determine the location and mechanism of an inherited retinal degeneration using as a model system rod-cone dysplasia in the Irish setter. This is one of a group of retinal degenerations collectively termed "progressive retinal atrophy". It was known from early experiments that cGMP levels were extremely high in affected whole retinas and since it is the outer segment (OS) layer that normally contains most of the cGMP, it was assumed that this was the location of the cGMP in this disease state also.

We have now shown that it is the opposite end of the photoreceptor cell, the outer plexiform layer, that contains the bulk of the retinal cGMP in diseased retinas. At the time of the peak levels (28 days), cGMP is 16-fold higher in diseased vs normal OPL. Levels remain high for at least 7 weeks before dropping, presumably as a result of photoreceptor cell degeneration.

Neither masked light or EM microscopy can detect changes between normal and dystrophic retinas before 13 days, yet we can see a clear difference in cGMP profiles across the retinal lyers as early as 11 to 12 days when there is a 6-fold difference in cGMP in the OPL. However, a much greater, 23-fold increase in cGMP levels in the OPL occurs in dystrophic retinas between 13 and 20 days, the time at which diseased retinas appear to be blocked in their normal differentiation process.

We are now focusing on measuring guanylate cyclase activity in retinal layers, particularly the OPL, to see if it is the increased activity of that enzyme that is responsible for the large increases in cGMP that we see.

This project has been terminated as of FY'85.

READEX FICHE

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-NS-02429-06 LNC

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Coordinate Changes in Brain Energy Metabolism and Protein Synthesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. I. : T. S. Nowak, Jr. Senior Staff Fellow LNC NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Neurochemistry

SECTION
Section on Cellular Neurochemistry

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	0.9	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Changes in brain energy metabolism and protein synthesis have been characterized following transient bilateral ischemia in the gerbil. Analysis of in vitro translation products by two dimensional gel electrophoresis has demonstrated the induction of the major mammalian stress-induced or heat shock protein, (hsp 70), during recirculation following 5 min ischemia. Expression of hsp 70 increases gradually following recirculation, reaching a maximum at approximately 8 hr recirculation, and returns to near control levels by 24 hr. In contrast, the synthesis of all other individual proteins examined fell dramatically following ischemia and returned to control levels in parallel with the recovery of overall incorporation activity. This transient induction of hsp 70 resembles the classical heat-shock response defined on other systems, although at no time does hsp 70 become a major translation product.

A low level of hsp 70 synthesis is detected in preparations from control brain, and the protein can be found in silver stained gels of control brain proteins. Preliminary immunohistochemical localization of hsp 70 indicates its presence in essentially all neuronal cell bodies.

Preliminary radioimmunoassay data have demonstrated a 30% depletion of the opiate peptide, dynorphin A, in gerbil cerebral hemispheres between 1 and 24 hr following 5 min ischemia. Beta-endorphin and met-enkephalin levels were not affected by ischemia, although the gerbil population used in these studies appears to be quite heterogeneous with regard to beta-endorphin levels in brain. These observations may be relevant to the conflicting reports of effects of opiate antagonists on stroke symptoms in this animal model.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02142-11 LNC

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cerebral Metabolism in Altered Metabolic States of the CNS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. I.	:	D. S. Heffez	Visiting Fellow	LNC	NINCDS
Others	:	T. S. Nowak, Jr.	Sr. Staff Fellow	LNC	NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neurochemistry, IRP, NINCDS

SECTION

Section on Cellular Neurochemistry

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The effect of nimodipine, a selective calcium channel blocker, on brain metabolism was studied during ischemia and recirculation in the gerbil. Nimodipine delayed the fall in ATP during the first minute of ischemia. This effect was significant in striatum and cortex, but not in hippocampus. Glucose levels after 5 min recirculation were significantly higher in striatum and cortex of nimodipine pretreated animals. These observations constitute the first demonstration of an effect of this class of drugs on brain metabolism. Nimodipine levels in brain were measured using a radioreceptor assay in which each brain homogenate provided the source of both binding site and competing ligand.

This project has been terminated.

Publications:

Heffez, D. S., Nowak, T. S., Jr., and Passonneau, J. V.: Nimodipine levels in gerbil brain following parenteral drug administration. J. Neurosurg., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-NS-01586-18 LNC

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Trophic Interactions of Neuronal and Target Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. I. :	A. A. Zalewski	Section Head	LNC	NINCDS
Others :	Y. Kadota	Visiting Associate	LNC	NINCDS
:	N. A. Azzam	Guest Researcher	LNC	NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Neurochemistry, IRP, NINCDS

SECTION
Section on Neuronal Development and Regeneration

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to determine factors which aid nerve fiber regeneration. In the present study we have examined the binding pattern of fluorescin-labeled lectins to nerve. Lectins are substances which bind specifically to the sugar portion of glycoconjugates and therefore can be used to detect cell surface and extracellular matrix molecular changes that occur during nerve degeneration and regeneration. In normal rat nerve, the lectins Concanavilin, Maclura and Wheat germ bound to the perineurium and basement membrane of endoneurial Schwann cells and blood vessels. Soybean agglutinin stained the endothelium throughout the nerve. Dolichos, Peanut and Griffonia attached only to the perineurium. Because of a turnover in laboratory personnel this experiment was interrupted. However, with arrival of new investigators, the binding of lectins during peripheral nerve regeneration will be undertaken and observations extended to the central nervous system. We especially want to examine the transition zone between the peripheral and central nervous system to determine why regeneration ceases at this region when nerve grafts are used to promote nerve fiber regeneration.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-NS-02254-09 LNC

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Repair of Injured Nerve With a Nerve Allograft

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. I.	:	A. A. Zalewski	Section Head	LNC	NINCDS
Others	:	Y. Kadota	Visiting Associate	LNC	NINCDS
	:	N. A. Azzam	Guest Researcher	LNC	NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Neurochemistry, IRP, NINCDS

SECTION
Section on Neuronal Development and Regeneration

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.7	1.8	0.9

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although nervous tissue allografts survive during treatment with the immunosuppressive agent Cyclosporin-A (Cy-A) they are rejected once therapy is stopped. Accordingly, we have begun to explore ways in which the antigenicity of the allograft might be eliminated or the host rendered tolerant to the antigens of the allograft. In one study donor rats were perfused with a physiological salt solution to remove blood from their nerves prior to transplantation. Since blood alone can evoke an immune reaction, this manipulation was expected to alter allograft antigenicity. However, despite removing the blood, the nerve allografts were rejected. The perfusion procedure did not alter the viability of the grafts since they survived and underwent Wallerian degeneration in genetically compatible (i.e., isogeneic) hosts. In another study, nerve allografts, resident for 3-5 months in immunosuppressed rats, were retransplanted into non-immunosuppressed hosts. These retransplanted nerves were also rejected indicating again that allogeneic blood alone is not responsible for inciting rejection and that nerve allograft cells retain their antigenicity, even after prolonged periods of transplantation. In an attempt to induce tolerance to allograft antigens, host rats were given donor blood intravenously, at the time of nervous tissue grafting, and maintained on Cy-A for only one week. Interestingly, allogeneic neurons survived after three months whereas nerve allografts were rejected. We plan to investigate the reason for these divergent results as well as to improve upon our use of blood and Cy-A as a tolerogenic protocol.

Data from other studies indicated that hamster neurons survived in the spinal cord of Cy-A immunosuppressed rats. If this result applies to other species, it suggests that xenogeneic neurons can be used clinically.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-NS-00813-24 LNC

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Enzymological Aspects of Neural Functions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. I.	:	R. W. Albers	Chief, Sec. on Enzyme Chemistry	LNC	NINCDS
Others	:	S. P. Chock	Expert Consultant	LNC	NINCDS
	:	A. K. Hazra	Visiting Associate	LNC	NINCDS
	:	P. M. Rowe	Staff Fellow	LNC	NINCDS

COOPERATING UNITS (if any)
J. P. Froehlich, NIA, NIH, Baltimore
R. H. Huang, Univ. Mo. Health Sci., Kansas City
T. S. Nowak, Section on Cellular Neurochemistry, LNC, NINCDS

LAB/BRANCH
Laboratory of Neurochemistry, IRP, NINCDS

SECTION
Section on Enzyme Chemistry

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.9	2.2	1.7

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project comprises an investigation of the structure and functioning of the sodium pump. Rapid quenching studies have shown the presence of an "intermediate" component of the dephosphorylation rate curve in the memrane-bound enzyme from Electrophorus electric organ. We have developed two different preparations of soluble Na,K-ATPase. One of these preserves the membrane-type dephosphorylation kinetics, whereas the other type displays monophasic dephosphorylation. Disappearance of the intermediate rate component is correlated with a doubling of catalytic activity. Since molecular seiving and cross-linking studies indicate that both preparations consist of monodisperse associations of 3-4 enzyme protomers, the provisional hypothesis for current studies is that the native enzyme exists as an oligomer and that modification of oligomeric interactions by the solubilizing detergent is responsible for the altered kinetics. Other studies to correlate these differences with physical and other biochemical parameters of the Na,K-ATPase and to determine the transport competence of the two forms are in progress.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02605-02 LNC

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of Mast Cell Secretion and Membrane Generation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. I.	:	Stephen P. Chock	Expert	LNC	NINCDS
Others	:	R. W. Albers	Acting Chief	LNC	NINCDS

COOPERATING UNITS (if any)

E. A. Schmauder-Chock, Department of Experimental Hemotology, Armed Forces Radiology Research Institute (AFFRI)

LAB/BRANCH

Laboratory of Neurochemistry, IRP, NINCDS

SECTION

Section on Enzyme Chemistry

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	0.9	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The role of histamine in anaphylactic reaction is well established. Information concerning the role of histamine in modulating neuronal and cellular functions through specific H_1 and H_2 histamine receptors are now accumulating. The study of the mechanism of histamine release from rat peritoneal mast cells will enable us to understand the mechanism of neuropeptide secretion and the mechanism by which eukaryotic membrane assembly can occur.

The generation of de novo membrane has been shown to occur following granule activation in rat peritoneal mast cells (Chock, S.P. and Chock, E.A. Fed. Proc. 44, 1324 (abstr. 5341), 1985). The rapid assembly of this new membrane may provide a model for studying membrane formation in eukaryotic cells.

We have localized a high level of calcium and a calmodulin-like activity in the granule matrix. The role of calcium and calcium-binding protein in promoting membrane fusion has been suggested before. Their presence here might be to enhance the fusion of the newly assembled membrane with the plasma membrane and thus facilitate exocytosis.

In order to firmly establish our hypothesis that de novo membrane generation occurs during granule activation, we will attempt to demonstrate that the swelling of the granule matrix and the expansion of the perigranular membrane occurs prior to the fusion of the perigranular membrane with the plasma membrane. It is also important to be able to isolate unactivated quiescent granules for phospholipid determination such that the source of the new membrane can be verified.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02631-02 LNC

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function in Retinal Neurons

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. I.:	Ralph Nelson	Physiologist	LNP, NINCDS
Others:	Helga Kolb	Prof., Dept. Physiol.	University of Utah
	Andrew P. Mariani	Senior Staff Fellow	LNP, NINCDS
	Renata Pflug	Visiting Associate	LNP, NINCDS
	Michael Freed	Guest Researcher	LNP, NINCDS
	Jan Nora Moura de Melo	Guest Researcher	LNP, NINCDS
	Keith Purpura	Guest Researcher	LNP, NINCDS

COOPERATING UNITS (if any)

Department of Physiology, University of Utah, Salt Lake City (H. Kolb)

LAB/BRANCH

Laboratory of Neurochemistry, IRP, NINCDS

SECTION

Neural Circuitry Unit

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL: *	OTHER:
0.7	0.0	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been transferred to the Laboratory of Neurophysiology.*

*[Professional support man-years were performed under LNP.]

PHS 6040 (Rev. 1/84)

GPO 914-918

TAB 10 -- LABORATORY OF NEURO-OTOLARYNGOLOGY -- (LNO)

ANNUAL REPORT

October 1, 1984 through September 30, 1985

Laboratory of Neuro-otolaryngology

National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT
October 1, 1984 through September 30, 1985
Laboratory of Neuro-otolaryngology, IRP
National Institute of Neurological and
Communicative Disorders and Stroke

Jörgen Fex, M.D., Ph.D., Chief

The Laboratory has continued to provide new knowledge within the framework of its two Projects: Project Number Z01NS02216-10 LNO, Inner Ear Neuronal Mechanisms: A Multidisciplinary Analysis, Project number Z01 NS02217-10 LNO: Synaptic Transmission and Neuronal Connections of the Mammalian Cochlear Nucleus. Through these Projects we aim at a better understanding of how the inner ear can make us hear and how the cochlear nucleus processes the auditory information that it receives from the inner ear.

We are particularly stimulated by our findings that isolated outer hair cells when electrically stimulated give responses that are not dependent on metabolically based energy. These responses could be visualized only through the use of video enhanced microscopy. We have excellent evidence for the presence of an electro-osmotic mechanism as an explanation for the shape changes of outer hair cells that was seen. This type of phenomenon, not previously described, could be importantly involved in the function of outer hair cells as well as of other cells or cell components, such as dendritic spines where the structural configuration may provide conditions for electro-osmotic driven shape changes.

The olivocochlear neurons impinging on outer hair cells very likely play an important role in biasing the micromechanics of the organ of hearing through setting the outer hair cells towards greater or less sensitivity. We have contributed more than any other research team to the knowledge about this system and other efferent neurons in the cochlea. This year, through showing that axons and endings of efferent neurons in the organ of Corti contain GABA-like immunoreactivity with a distribution similar to that of GAD-like immunoreactivity, as shown in a previous study, we have strongly added to the evidence that certain efferent neurons projecting to the organ of Corti, some of them to outer hair cells, are GABA-ergic. The evidence indicates, also, that such a GABA-ergic population of neurons is not part of the lateral and medial efferent systems.

We have determined this year that dynorphin and an enkephalin are co-contained in lateral olivocochlear neurons, running counterwise to general assumptions that that these two groups of substances are always separate (manuscript in preparation).

For several years our laboratory has been studying neurotransmitters in the cochlear nucleus and we have made significant progress. However, during this past year, we have made enormous progress. A highly specific antibody against GABA has given us excellent visualization of GABAergic terminals in light microscopy studies. This antibody tolerates high levels of glutaraldehyde and therefore ultrastructure is beautifully maintained in the EM studies. Furthermore, our use of an antibody against the glycine receptor allows, for the first time, the visualization and identification of glycine synapses in the auditory system. These studies are an important step towards understanding the function of glycine and GABA in the normal and abnormal auditory system.

We have shown that both the cochlear nucleus and superior olivary complex have large and complex GABA and glycinergic inputs. These inhibitory inputs will be important factors in direct CN auditory prosthesis and future studies can address questions related to this.

Excitatory amino acids are believed to be major neurotransmitters in the CNS. In the auditory system, there is strong evidence that glutamate or aspartate is the neurotransmitter of the auditory nerve and of certain interneurons in the cochlear nucleus. Our immunocytochemical studies are intended to provide information on enzymes involved in the biosynthesis of the neurotransmitter pools of these amino acids, and perhaps define how excitatory amino acid neurons may be immunocytochemically marked. We have found that glutaminase is enriched in many such neurons but not all, while aspartate aminotransferase is enriched in only a few of these neurons. This suggests that there are biosynthetic pathways that are not present in all excitatory amino acid neurons. Our results suggest that varying combinations of glutaminase, aspartate aminotransferase, and perhaps other enzymes as well as high affinity, uptake play a role in the production of the neurotransmitter pools of these amino acids.

Approaching the issue of excitatory amino acids as neurotransmitters from another angle we have determined on tissue slice preparations that the auditory nerve postsynaptic receptor in chickens is of the kainate type, and in mammals it is of the NMDA type. It has also been established that it is possible to grow explant and dissociated cultures of auditory brainstem structures. The significance of these observations is considerable. It is now possible to consider studies on factors controlling the expression and regulation of excitatory amino acid postsynaptic receptors in the auditory system in particular, and the brain in general.

The goal of our biochemical research on the auditory nerve is to identify critical auditory nerve proteins and characterize them under normal and abnormal conditions. We have concentrated on two groups of proteins, the rapidly transported proteins (RTPs) and a group of proteins whose expression is changed after hair cell loss. Research this past year has focussed on the former group. RTP 1 is a major axonally transported fast component protein in the auditory nerve. That it is a major auditory nerve protein, degrades rapidly and may have several related forms as shown in our present studies, suggests that this protein may play a critical role in the function of the auditory nerve. We are particularly interested in determining chemical changes that occur in the auditory nerve after hair cell loss. This is a critical issue since the success of cochlear prostheses is dependent on a functioning auditory nerve. For example, it is known that spiral ganglion cells slowly die after hair cell loss and that eventually only a small population remain. We are trying to determine if the remaining neurons are biochemically normal, or if biochemical changes occur, perhaps to allow their survival.

We intend to continue to focus our attention on isolated auditory sensory cells under cell culture conditions. We will study these cells using video enhanced contrast optics. An important first step is to establish criteria of viability and survivability of the cells. If possible, these criteria will be defined exclusively in terms of observations given by the video light microscopy technique. We will study isolated cells of the organ of Corti using immunohistochemistry to establish good markers for different cell types in this organ.

We intend to try and create hybridomas between cells of the mouse cochlea and myeloma cells using electrofusion (Zimmermann-type). We have reasons to believe that this will be feasible. With such hybridomas in hand, we aim to create and study immortal cell lines.

We wish to continue to study the organ of Corti using immunocytochemical techniques. In particular, we are interested in the issue of co-containment at synapses of transmitter substance candidates and may pursue this at a cellular level, using immunoelectron microscopy.

We are coming below a minimal critical mass of personnel and may have to discontinue essential parts of our research projects. A series of extremely unfortunate circumstances have led up to this situation. This last spring, two foreign Ph.D.'s that were recruited to join the LNO found it not possible to come to the USA; the LNO is against expectations losing its morphologist, who has been with us for seven years, at the end of this fiscal year; a hiring freeze has blocked our attempt to add a technician to the LNO that we asked for this spring.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02216-10 LNO

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Inner Ear Neuronal Mechanisms: A Multidisciplinary Analysis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jörgen Fex	Chief	LNO, NINCDS
Others:	R. A. Altschuler	Senior Staff Fellow	LNO, NINCDS
	R. J. Wenthold	Chemist	LNO, NINCDS
	J. A. Rubio	Visiting Fellow	LNO, NINCDS
	M. J. Frye	Electronics Technician	LNO, NINCDS
	M. H. Parakkal	Bio Lab Tech (Micro)	LNO, NINCDS
	K. A. Reeks	Biologist	LNO, NINCDS
	J. M. Zempel	Biological Aid	LNO, NINCDS
	N. C. Jones	Student Volunteer	LNO, NINCDS

COOPERATING UNITS (if any) Laboratory of Neurobiology, NINCDS (B. Kachar); Laboratory of Clinical Science, NIMH (N. Zamir); Departments of Psychiatry and Pharmacology, Southern Illinois Univ., Springfield, IL (D. W. Hoffman); Dept. of Otolaryngology, Johns Hopkins Univ., Baltimore, MD (W. E. Brownell)

LAB/BRANCH

Laboratory of Neuro-otolaryngology

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.1	1.8	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to provide new knowledge of the auditory mechanisms of the inner ear.

We have found that mechanically dissociated and isolated outer hair cells of the organ of Corti of the guinea pig when electrically stimulated show shape changes that are not dependent on metabolically based energy. These changes could be visualized only through the use of video enhanced microscopy. There is evidence that an electro-osmotic mechanism was underlying these changes.

We have continued immunocytochemical studies, using small mammals. We have used several different antibodies, among them a highly specific antibody against GABA. We have studied the distribution of immunoreactivity through light microscopy, including video enhanced microscopy, and electron microscopy.

We have found that axons and endings of efferent neurons in the organ of Corti contain GABA-like immunoreactivity with a distribution similar to that of GAD-like immunoreactivity, as shown in a previous study, which strongly adds to the evidence that certain efferent neurons projecting to the organ of Corti, some of them to outer hair cells, are GABA-ergic. The evidence indicates, also, that such a GABA-ergic population of neurons is not part of the lateral and medial efferent systems.

We have determined that dynorphin and an enkephalin are co-contained in lateral olivocochlear neurons.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02217-10 LNO

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Synaptic Transmission and Neuronal Connections of the Mammalian Cochlear Nucleus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jorgen Fex	Chief	LNO, NINCDS
Others:	R. A. Altschuler	Senior Staff Fellow	LNO, NINCDS
	M. R. Martin	Pharmacologist (Research)	LNO, NINCDS
	R. J. Wenthold	Chemist	LNO, NINCDS
	M. J. Frye	Electronics Technician	LNO, NINCDS
	D. Huie	Chemist	LNO, NINCDS
	M. H. Parakkal	Bio Lab Tech (Micro)	LNO, NINCDS
	K. A. Reeks	Biologist	LNO, NINCDS
	J. M. Zempel	Biological Aid	LNO, NINCDS

COOPERATING UNITS (if any) Center for Molecular Biology, University of Heidelberg, Heidelberg, Federal Republic of Germany (H. Betz)

LAB/BRANCH

Laboratory of Neuro-otolaryngology

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 5.2	PROFESSIONAL: 2.4	OTHER: 2.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is to provide new knowledge of how the cochlear nucleus processes information coming from the organ of hearing through the auditory nerve.
Small mammals have been used in these studies.

A highly specific antibody against GABA, used by us for immunocytochemistry, has given us excellent visualization of GABAergic terminals in light microscopy studies. This antibody tolerates high levels of glutaraldehyde and ultrastructure is beautifully maintained in the EM studies. Furthermore, our use of an antibody against the glycine receptor allows the visualization and identification of glycinergic synapses in the auditory system. We have shown that both the cochlear nucleus and superior olivary complex have large and complex GABA and glycinergic inputs.

We have used antisera against glutaminase and aspartate aminotransferase in immunocytochemical studies to try and define how excitatory amino acid neurons may be immunocytochemically marked. We have found that glutaminase is enriched in many such neurons but not all, while aspartate aminotransferase is enriched in only a few of these neurons.

We have determined on tissue slice preparations that the auditory nerve postsynaptic receptor in chickens is of the kainate type, and in mammals it is of the NMDA type.

We have biochemical studies in progress of axonally transported proteins in the auditory nerve, concentrating on two groups of proteins, the rapidly transported proteins (RTPs) and a group of proteins whose expression is changed after hair cell loss. This study relates to the critical issue that the success of cochlear prostheses is dependent on a functioning auditory nerve.

TAB 11 -- LABORATORY OF NEUROPATHOLOGY AND NEUROANATOMICAL SCIENCES -- (LNNS)

ANNUAL REPORT

October 1, 1984 through September 30, 1985

Laboratory of Neuropathology and Neuroanatomical Sciences
National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT
October 1, 1984 through September 30, 1985
Laboratory of Neuropathology and Neuroanatomical Sciences, IRP
National Institute of Neurological and Communicative Disorders and Stroke

Igor Klatzo, Chief

The Laboratory of Neuropathology and Neuroanatomical Sciences (LNNS) has continued within its three sections (Section on Cerebrovascular Pathology, Section on Cerebrovascular Physiology and Section on Neurocytology) the research focussed on elucidation of various aspects of pathophysiology of cerebrovascular disorders in order to get a better understanding of mechanisms involved and to develop basis for application of rational therapeutic measures.

The Section of Cerebrovascular Pathology has continued further elucidation of interrelationship between the thresholds of cellular injury and selective vulnerability in cerebral ischemia.

The different thresholds of neuronal injury at given levels of reduction of cerebral blood flow (CBF) were demonstrated not only with regard to different anatomical brain structures but also with regard to the age of an experimental animal; e.g. 3 week-old gerbils showing remarkable resistance to ischemic injury in comparison to adult (3 month-old) animals. Although the impact of ischemia, reflected in intermittent measurements of CBF by hydrogen clearance, was similarly severe in various brain regions in both adult and young gerbils subjected to 5 minute ischemia, during the post-ischemic periods the adult animals revealed a marked CBF hypoperfusion in hippocampus which showed later evidence of morphological damage, whereas young animals revealed no post-ischemic hypoperfusion and no damage in the hippocampus. Also, oxygen availability determinations in hippocampus indicated at 30 and 60 minute recirculation a tissue hypoxia much more pronounced in the adult than in the young gerbils. These studies indicate that among factors accounting for better tolerance of ischemia by young gerbils, in addition to previously demonstrated slower energy utilization during initial stage of ischemia, considerably less pronounced tissue hypoxia during post-ischemic periods, as indicated by CBF and oxygen availability determinations, may play a significant role in pathomechanisms of ischemic injury.

The different metabolic response concerning different structures and age of the animals was evident with regard to behavior of glycogen which is related to glucose metabolism. Following 5 minute ischemia there was a rapidly progressive accumulation of glycogen in various structures, most pronounced at 6 hours after ischemic insult. This was followed by a striking reduction in glycogen, especially in the hippocampus which was observed at 24 hours. A maximum accumulation of glycogen, especially in Schaffer's collaterals was seen at 48 hr post-ischemic time interval. These observations on glycogen seem to correlate with our previous studies on neuronal activity extracellular recordings in gerbils subjected to 5 minute ischemia. The current observations on glycogen indicate that periods of neuronal hyperactivity is associated with a conspicuous reduction of glycogen, whereas the collapse of neuronal activity, demonstrated previously at 48 hours, corresponds to a striking accumulation of glycogen, primarily in astrocytic cells.

In elucidating the role of edema in pathophysiology of ischemic injury, in addition 1) to establishing a direct quantitative relationship between extravasation of proteins and water retention in the tissue, and 2) to demonstrating the beneficial effect of prevention of exudation of proteins on the course of ischemic lesions, our recent studies demonstrated a new transvascular route for removal of extravasated proteins which can contribute to resolution of vasogenic edema. Ultrastructural observations, using horseradish peroxidase (HRP) as the tracer, revealed a pinocytotic uptake of the HRP injected 24 hours earlier by endothelial cells of arterioles and venules in the area of vasogenic edema in which there was no demonstrable evidence of abnormal uptake or transendothelial transport of the tracer from the luminal side. Otherwise, in ischemia and in epileptic seizures we have observed a ready uptake of extravasated proteins from the extracellular space into the neurons. As much as in ischemia intracytoplastic uptake of proteins reflects various degrees of cell membrane injury, our current studies in bicuculline-induced seizures revealed that the intraneuronal entry of extracellular HRP takes place by vesicular uptake into presynaptic boutons, which potentially may play a significant role in further propagations of the seizures.

The presence of "semi-viable" neurons containing extravasated serum proteins was observed in our recent studies several days after ischemic insults with intensities around the threshold levels (e.g. 20 minute middle cerebral artery occlusion in cats), as well as in areas of penumbra surrounding foci of severe necrotic ischemic injury. Such neurons seem to represent a new, "chronic" phase of ischemic injury and it is under current investigation whether such neurons may be hyper-active and lead to development of epileptogenic foci or to further propagation of the ischemic lesions.

The Section on Cerebrovascular Physiology has been involved in a study of the effects of focal ischemia of the brain upon cerebral extracellular space determined by impedance measurements. Cats were subjected to unilateral middle cerebral artery occlusion (MCAO) for variable periods of time. The cerebral electrical impedance (CEI), measured by a platinum electrode array implanted in the selected region of the brain, was followed throughout the experiment, as was the regional cerebral blood flow (rCBF) and other physiological parameters. During the period of ischemia the rCBF fell to less than 12 ml/100g/min, the CEI climbed quickly within a few minutes to 150% or more. The CEI usually but not always continued to rise but much more slowly. (This indicated that a second process different from that previously described was present). When we made the duration of occlusion relatively short (1 hr or less), the CEI reached a stable plateau value of 200% or more of the base line value. Upon release of the occlusion, the impedance fell abruptly and rapidly within less than a minute reaching the base line value asymptotically in about 1 hr. During this phase the rCBF initially became hyperemic but returned quickly to base line flows in less than 1 hr. The intracranial tissue pressure was not particularly disturbed during these phases even though substantial changes in the CEI had occurred. When the ischemic duration was made longer than about 1 hr we began to see more complex changes, including further increases in the CEI, and in the intracranial tissue pressure indicating severe edema development. It is planned to study the dynamics of the CEI in long term MCAO (durations greater than 1 hr) and it's relationship to the other physiological parameters including edema.

We have focused our attention on the development of edema to ascertain how it maybe reflected in impedance measurement. With MCAO as brief as 20 minutes, we found detectable extravasation's into grey matter of Na fluorescein and/or Evans Blue which had been injected to test whether the Blood-Brain Barrier (BBB) leaked into grey matter over the first few hours. This evidence of BBB leakage was coupled to specific gravity measurements of the brain tissue in the same areas that showed leakage. The peak intensity of edema was observed to be between 1/2 and 1 hr. Following this the edema was less severe with complete resolution at 6 hours. As described above, the CEI following release from occlusion returned to pre-ischemic levels and below, quickly within 10 minutes after a 20 minute MACO.

These observations showing the return of CEI to normal values at the time when water content of the brain tissue, previously subjected to ischemia, remains elevated indicates that there is restoration of the size of extracellular spaces in spite of the cytotoxic swelling of the cells which develops promptly after the onset of ischemia. The mechanisms of such restoration, its relationship to increased vascular permeability or the osmotic changes within the extracellular compartment are the subject of current investigations within the section.

In collaboration with Professor Thomas Devlin of Hahnemann Medical College and Hospital in Philadelphia, Pa. an effort was made to evaluate a prostaglandin derivative called PGBx for its protective action against ischemic brain damage. It was reported that PGBx restored oxidative phosphorylation in mitochondria following exposure to severe hypoxia. Our effort began with a test of the efficacy of PGBx in a model of 15 minute bilateral cerebral ischemia in gerbils, developed in this laboratory. While the initial results showed favorable survival rates, further tests of the drug did not confirm the earlier results. Our findings do not necessarily invalidate reported results such as the effect of PGBx on oxydative phsophorylation in mitochondria or other metabolic studies, however, in view of the failure to demonstrate the beneficial effects in this model, further study of this substance has been suspended.

The continuous goals of the Section on Neurocytobiology have been: I) to develop and utilize new model systems for the investigation of basic mechanisms operative on the level of normal and pathologically altered blood-brain barrier (BBB) and cerebral blood flow (CBF); II) to study the metabolic processes occurring in cerebral ischemia and ischemic edema, especially their prevention and therapy.

I. During the last years both the newly established pure muscle cell culture (Spatz et al. Brain Res.) 280: 387-391, 1983 and the previously developed endothelial culture derived from dissociated cerebral microvessels (Spatz et al. Brain Res.) 191: 577, 1980, have been very useful models for the continuous studies of cerebrovascular function related to the BBB, CBF and SBP.

Four different aspects related to the cerebral capillary function in vivo have been investigated in the in vitro models using the pure cerebrovascular endothelial and/or smooth muscle cell culture: a) Endothelial permeability b) Effects of vasoactive substances on carbohydrate metabolism c) The synthesis of prostaglandins and its stimulation by various hormones implicated in the regulations of events occurring on the level of BBB, interface.

a) Cerebrovascular endothelial 'barrier' function (endothelial permeability) was investigated under controlled conditions in an in vitro model. The cells were grown to confluency on dextran microbeads which accumulate small molecular weight dyes at a rate determined by the permeability of the cells covering the beads. Exposure to hypertonicity (400 mosm/l) as well as high concentrations of arachidonic acid (100 μM) caused a breakdown of the 'barrier', whereas 10μM of arachidonic acid increased permeability only in the presence of indomethacin (10μM). High concentrations of indomethacin (100μM) enhanced endothelial permeability to trypan blue without addition of arachidonic acid while ibuprofen (10-40 μM) failed to show similar changes. The results suggest that prostaglandin metabolism and/or synthesis may participate in altering endothelial barrier function in pathological states.

b) The separately cultered smooth muscle and endothelial cells derived from dissociated cerebral microvessels are characterized by high content of glycogen. Norepinephrine induces glycogenolysis while 5-hydroxytryptamine stimulates glycogenesis in both cell types. The endogeneous glucose of the endothelium but not that of the smooth muscle serves as a direct source for the 5-HT enhancement of glycogen formation. Indomethacin, the known inhibitor of cyclooxygenase modulates the glycogen content in the smooth muscle only. These findings strongly suggest that the carbohydrate metabolism of each cell has a distinct control mechanism compatible with the underlying integral microvascular function.

c) The sites of possible cellular prostaglandin interaction with angiotensin and kinins (which could play a role in regulating local microcirculation and/or blood pressure) have been investigated in separately cultured cerebrovascular endothelium and smooth muscle, and compared their responses to that in the glia. The greatest stimulation of prostaglandin release was observed in the medium of the smooth muscle cells exposed to either angiotensin (I or II) or to bradykinin. (The concentrations of 6-keto-PGF$_{1}\alpha$ were up to 100 times over the basal levels). The synthesis of prostaglandin in endothelium and glia was not significanntly affected by the addition of angiotensin I. However, a slight enhancement of prostaglandin release from each cell line was observed after incubation with either angiotensin II or bradykinin (20-30% over basal level). Both, the lack and the low response of prostaglandin synthesis to the tested peptides in these cells in contrast to that of smooth muscle might be due to and enzymatic (kinase II) degradation of angiotensin I and bradykinin in the former but not in the latter cells. These results strongly indicate that the cerebromicrovascular smooth muscle cells represent the most sensitive site for prostaglandin-peptide interaction which maybe responsible for the modulation of vascular reactivity. The less responsive synthesis of prostaglandin to angiotensin and bradykinin observed in endothelium and glia suggests that these cells might serve as protectors of smooth muscle by inactivating peptides or by other mechanisms. Thus, each of the cells might have an influence on the cerebral microcirculation through its distinct and interrelated actions.

In addition, the collaborative studies with Drs. McCarron and McFarlin (Neuroimmunology Branch) revealed that cerebromicrovascular endothelium (murine) can present guinea pig basic protein in a 1-A restricted manner similar to that observed in macrophages. Thus, the cerebral capillaries provide a mechanisms by which lymphocytes may transgress the BBB and initiate EAE in animals.

II. a) The development and progression of ischemic cerebral edema have been studied in respect to changes in serotonin (5-HT) metabolism. Recently we detected in the cerebro-cortical homogenate a modulation of S_2(postsynaptic 5-HT) receptor binding sites coinciding with a attenuated metabolic rate of 5-HT (= increased release) and accumulation of water on the tissue. To ascertain more closely the relationship of the postsynaptic S_2-receptors changes to 5-HT release and edema formation, we have investigated the properties of these receptors in synaptosomes obtained from obviously edematous and unedematous brains. (Cerebral ischemic edema was induced by 15 min, but not by 5 ischemia and 1 hour recirculation).

The alteration of ^{3}H-ketanserin (the potent 5-HT antagonist which labels specifically S_2-receptor sites) bindings sites in synaptosomes were found in association with an increased release of 5-HT and accumulation of water in the brain after 15 min of ischemic insult only. Thus, these findings support the implicated serotoninergic participation in pathogenesis ischemic brain edema.

b) The studies on cerebral ischemia, its pathophysiology, prevention and therapy in gerbils have been concerned with continous evaluation of the effects of naturally occurring central nervous system depressant [-butyrolactone (GBL) and -hydroxybutyrate (GHB)] on cerebral ischemia focusing on the elucidation of the possible mechanisms responsible for the observed beneficial effect of GBL and GHB on ischemic brain edema. These investigations revealed that the postischemic GHB treatment 3 hours after release of bilateral carotid occlusion reversed the observed increased turnover time and decreased turnover rate (decreased 5-HT release and synthesis) in the cortex, hippocampus and partly in the striatum. However, 5-HIAA accumulation was unaffected by GHB treatment.

Nevertheless, these findings suggest that the reported beneficial manifestations of the postischemic GHB treatment might be related to 5-HT synthesis which appears to be enhanced in the treated as compared to untreated ischemic animals. These results confirm our previous observation that the post-ischemic GHB treatment stabilizes the ischemically disturbed serotonin metabolism. Therefore, the observed short term improvement in one of the monoamines metabolism following a relatively late postischemic treatment warrants further studies of GHB as a potential therapeutic agent in cerebral ischemia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02275-09 LNNS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Prostaglandin synthesis (PGI_2) in cultured cerebromicrovascular elements and glial cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

M. Spatz, Head, Section on Neurocytobiology, LNNS, NINCDS
B. Wroblewska, Visiting Fellow, LNNS, NINCDS
O. Kempski, Visiting Fellow, LNNS, NINCDS

COOPERATING UNITS (if any)

L.S. Wolfe, Montreal Neurological Inst. McGill Univ. Montreal CA.

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	.2	.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The cellular prostaglandin interaction with angiotensin and kinins has been considered of great importance in the regulation of systemic blood pressure and possibly in local regulation of the brain microcirculation. Therefore, we investigated the effect of angiotensin (I or II) or bradykinin on prostaglandin synthesis in separately cultured cerebrovascular endothelium and smooth muscle, and compared their responses to that in the glia.

The synthesis of PGI_2 was measured indirectly in the medium of each cell type using radioimmunoassay with $[^{125}I]$ 6-keto-$PGF_1\alpha$.

The greatest stimulation of prostaglandin release was observed in the medium of the smooth muscle cells exposed to either angiotensin (I or II) or to bradykinin. (The concentrations of 6-keto-$PGF_1\alpha$ were up to 100 times over the basal levels). The synthesis of prostaglandin in endothelium and glia was not significantly affected by the addition of angiotensin I. However, a slight enhancement of prostaglandin release from each cell line was observed after incubation with either angiotensin II or bradykinin (20-30% over basal level).

These results strongly indicate that the cerebromicrovascular smooth muscle cells represent the most sensitive site for prostaglandin-peptide interaction which maybe responsible for the modulation of vascular reactivity. The less responsive synthesis of prostaglandin to angiotensin and bradykinin observed in endothelium and glia suggests that these cells might serve as protectors of smooth muscle by inactivating peptides or by other mechanisms. Thus, each of the cells might have an influence on the cerebral microcirculation through its distinct and interrelated actions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02324-08 LNNS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Blood-brain barrier: In vitro model for the study of cerebrovascular endothelial permeability

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

O. Kempski, Guest Worker, LNNS, NINCDS
M. Spatz, Head, Section on Neurocytobiology, LNNS, NINCDS

COOPERATING UNITS (if any)

None

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Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.6	1.6	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies of pathophysiological mechanisms of blood-brain barrier (BBB) disturbances have been hampered by the functional interrelationship existing between various cerebral elements and particularly by the simultaneous changes of many parameters occurring during pathological events in-vivo. An in-vitro model has been developed to investigate 'barrier' functions in cultured cerebromicrovascular endothelium under controlled conditions.

Changes in endothelial peremability were tested by exposing the cells to 1) hypertonic solution (known to alter the BBB permeability in-vivo), and 2) agents which have been implicated in mediating brain edema.

1) Hypertonic solution (400 mosm/l) increased significantly the endothelial permeability to trypan blue without apparent decrease of cellular viability when compared to isotonic medium. 2) High concentration of arachidonic acid [(100μM), the prostaglandin precursor] almost completely disrupted the endothelial 'barrier' while lower levels of this substance (10μM) increased the cellular permeability to trypan blue only in the presence of indomethacin (10μM), the known inhibitor of prostaglandin synthesis. However, indomethacin alone in high concentrations (100μM) also enhanced the endothelial permeability to trypan blue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02357-07 LNNS

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Experimental cerebral ischemia in mongolian gerbils: gamma-hydroxybutyrate effects on cerebral serotonin metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

K. Kumami, Visiting Fellow, LNNS, NINCDS
M. Spatz, Head, Section on Neurocytobiology, LNNS, NINCDS

COOPERATING UNITS (if any)

B.M. Djuricic, Biochemical Institute University of Belgrade, Yugoslavia

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Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.8	.1	.7

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The studies on cerebral ischemia, its pathophysiology, prevention and therapy in gerbils have been concerned with continuous evaluation of the effects of naturally occurring central nervous system depressant [-butyrolactone (GBL) and -hydroxybutyrate (GHB)] on cerebral ischemia focusing on the elucidation of the possible mechanisms responsible for the observed beneficial effect of GBL and GBH on ischemic brain edema. These investigations showed that the postischemic GHB treatment 3 hours after release of bilateral carotid occlusion GHB reverses the observed increased turnover time and decreased turnover rate (decreased 5-HT release and synthesis) at 4 hours reflow after the ischemic insult in the cortex hippocampus and partly in the striatum. However, 5-HIAA accumulation is unaffected by GHB treatment. These results suggest that the reported beneficial manifestations of the postischemic GHB treatment maybe related to 5-HT synthesis which appears to be enhanced in the treated as compared to the untreated ischemic animals. These findings also indicate that the postischemic GHB treatment stabilizes the ischemically disturbed serotonin metabolism. Therefore, the observed short term improvement in one of monoamines metabolism following a relatively late post-ischemic treatment warrants fruther studies of GHB as a potential theraputic agent in cerebral ischemia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02548-04 LNNS

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Evaluation of electrical impedance in cerebral ischemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

H.G. Wagner, Chief, Section on Cerebrovascular Physiology, LNNS, NINCDS
K. Kito, Visiting Fellow, LNNS, NINCDS
M. Seida, Visiting Fellow, LNNS, NINCDS
I. Klatzo, Chief, LNNS, NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Cerebrovascular Physiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 1.2	**PROFESSIONAL:** 0.9	**OTHER:** .3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

AN EVALUATION OF CEREBRAL ELECTRICAL IMPEDANCE (CEI) IN FOCAL CEREBRAL ISCHEMIA PRODUCED BY OCCLUSION OF THE MIDDLE CEREBRAL ARTERY (MCAO) FOR VARYING PERIODS OF TIME

A series of cats were subjected to MCAO. During the ischemia, the regional cerebral blood flow (rCBF) generally fell to less than about 12 ml/100g/min. The CEI rose rapidly within 3-4 minutes and reached 150% or more within 10 minutes. Following the rapid rise phase, a second phase of increase in impedance occurred but at a much slower rate. Upon release of the occlusion, the impedance begun to fall within less than a minute. This drop was precipitous at first, but more gradual with time. If the duration of ischemia was short (less than 1 hr) the CEI recovered to the base control value. In longer duration occlusions (1 hr or more) the CEI appeared to not recover completely and often trended up. This secondary rise was considered to be related to brain compression produced by brain edema.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02572-03 LNNS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effect of abolition of BBB opening on water content of ischemic brain tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. Ting, Special Expert, LNNS, NINCDS
T. Kuroiwa, Visiting Fellow, LNNS, NINCDS
I. Klatzo, Chief, LNNS, NINCDS

COOPERATING UNITS *(if any)*

None

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Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Cerebrovascular Pathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	.6	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The effect of prevention of reactive hyperemia, which invariably follows release of arterial occlusion in areas of the brain subjected to ischemia of intensity below threshold level, was evaluated with regard to opening of the blood-brain barrier (BBB) associated with extravasation of serum proteins, and to development of ischemic brain edema. The reactive hyperemia was abolished by hypovolemic withdrawal of the blood at the time of recirculation. Such animals showed no opening of the BBB to proteins significantly reduced edema, when tested at 3 hours following recirculation, in comparison to edema in normovolemic animals subjected to similar intensity of one hour ischemia. Brain injury determined at 3 days after recirculation varied from none to moderate in cats with severe ischemia (below 12 mg/100g/min) in which reactive hyperemia and opening of the BBB was prevented by hypovolemia, whereas the cats with similar in severity ischemia, accompanied by reactive hyperemia and extravasation of EB, revealed a frank cerebral infarction. These studies demonstrate further the significance of serum protein extravasation in the development of brain edema and with regard to severity of ischemic injury.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02576-03 LNNS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cerebrovascular smooth muscle cultures: Binding studies of α_2-adrenergic receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

B. Wroblewska, Visiting Fellow, LNNS, NINCDS
M. Spatz, Head, Section on Neurocytobiology, LNNS, NINCDS

COOPERATING UNITS (if any)

None

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Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	.8	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The presence α_2-adrenergic receptors was investigated in smooth muscle cell cultures using ^{3}H clonidine (α_2-adrenergic agonist) as a ligand.

Specific binding sites of ^{3}H clonidine were defined as the excess over "blank" taken in the presence of 1μm "cold" clonidine. For the competitive studies different concentration of various adrenergic agonists and antagonists were used to displace binding with 4nM ^{3}H-clonidine.

The rank order of potency for α-adrenergic agonists and antagonists was clonidine > phentolamine = yohimbine >> prazosin. The IC_{50} for the investigated displacers were: 25nM, 300 nM, 1μM and 9mM respectively. Competition curve produced by competing "cold" for ^{3}H-clonidine (4nM) showed a biphasic pattern indicative of multiple binding sites. Besides, the Scotchard analysis of saturation curve (concentration of ^{3}H-clonidine ranged from 6μM to .3 nM) and dissociation rate were characteristic of multiple population of the α_2-adrenergic binding sites in cultured smooth muscle cells. Thus, the existence of α_2-type adrenergic receptors not linked to AC activity observed in the cerebrovascular smooth muscle cells strongly suggest that their reactivity which is mediated by these receptors might be associated with Ca^{++} fluxes.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02620-02 LNNS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Reactivity of young gerbil brain to cerebral ischemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

H. Martinez, Visiting Associate, LNNS, NINCDS
R. Cahn, Visiting Fellow, LNNS, NINCDS
B. B. Mrsulja, Visiting Scientists, LNC, NINCDS
I. Klatzo, Chief, LNNS, NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cerebrovascular Neuropathology

SECTION

Section on Cerebrovascular Pathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Effects of 5 minute cerebral ischemia due to bilateral occlusion of the common carotid arteries were studies in 3 week old and adult gerbils. The biochemical assays revealed a considerable difference concerning the rate of depletion of the main energy metabolites indicating a slower energy metabolism in the young gerbils. Morphological studies carried out after 2 weeks revealed no evident ischemic injury in the young animals, whereas the brain of adult gerbils showed characteristic severe detruction of the CA1 sector of the hippocampus. The evaluation of regional cerebral blood flow (rCBF) with ^{14}C iodoantipyrine radioautography revealed severe, uniform (below 10 ml/100g/min) ischemia in most of the both hemispheres, similar in intensity in both young and adult gerbils. The quantitative rCBF measurements based on hydrogen clearance and using implanted platinum electrodes indicated a marked hypoperfusion demonstrable at 30' and 1 hr recirculation periods in adult animals, whereas this was not present in young gerbils. Oxygen availability determinations indicated at 30 and 60 minutes recirculation a tissue hypoxia, much more pronounced in the adult than in the young animals. The studies indicate that among factors accounting for better tolerance of ischemia by young gerbils, in addition to slower energy utilization during initial stages of ischemia, considerably less pronounced tissue hypoxia during post-ischemic periods may play a significant role in pathomechanisms of ischemic injury.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02622-02 LNNS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The effects of hypoosmotic solutions on cultured cerebrovascular endothelium

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

O. Kempski, Visiting Fellow, LNNS, NINCDS
M. Spatz, Head, Section on Neurocytobiology, LNNS, NINCDS

COOPERATING UNITS *(if any)*

G. Valet, Max-Planck Inst. Biochem. Martinsried FRG
A. Baethmann, Inst. Surg. Res. Univ. Munich, FRG

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	.1	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
  ☐ (a1) Minors
  ☐ (a2) Interviews
☐ (b) Human tissues
☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The cerebral microvessels, the active constituents of blood-brain barrier (BBB) posses many complex properties which are engaged in ensuring an optimal hoemostatically controlled environment for the brain. Since the capillary endothelial ability to regulate its volume should be prerequisite for maintaining the function of the barrier, we investigated the response of intrinsic endothelial mechanisms to hypotonicity.

The exposure of viable endothelium to a medium of half normal osmolalty resulted in immediate cellular swelling, reduction in transmembraneous potential and intracellular pH but without evidence of permeability changes to trypan blue bound proteins. A rapid recovery with complete normalization of cell volume and membrane potential but with limited restoration of intracellular pH took place within 30-60 minutes although the osmolality of the medium remained low. These results strongly suggest that the cerebrovascular endothelium has a build-in high capacity for selfregulation which undoubtfully is important for normal function of BBB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02623-02 LNNS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Serotonin(S_2)-receptors in ischemic brain edema

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

B. Wroblewska, Visiting Fellow, LNNS, NINCDS
K. Kumami, Visiting Fellow, LNNS, NINCDS
M. Spatz, Head, Section on Neurocytobiology, LNNS, NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The development and progression of ischemic cerebral edema have been associated with changes in serotonin (5-HT) metabolism. The present study represents a continuous effort to shed more light on the pathomechanisms of ischemic cerebral edema. These investigations centered to ascertain more closely the relationship between the changes of postsynaptic S_2-receptors in synaptosomes and the degree of cerebral of edema formation induced by ischemia of different duration.

Mongolian gerbils subjected to 5 or 15 min. of bilateral common carotid artery occlusion with and without 1 hr release served as the model for this study.

The alteration of ^{3}H-ketanserin (the potent 5-HT antagonist which labels specifically S_2-receptor sites) binding sites in synaptosomes was found in association with an increase release of 5-HT and accumulation of water in the brain after 15 min of ischemic insult only. Thus, these findings substantiate the implicated serotoninergic participation in pathogenesis of ischemic brain edema.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02625-02 LNNS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Efficacy of PGBx to protect against cerebral ischemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

H.G. Wagner, Chief, Section on Cerebrovascular Physiology, LNNS, NINCDS
B.B. Mrsulja, Visiting Scientist, LNC, NINCDS
H. Martinez, Visiting Fellow, LNNS, NINCDS
H. Masaoka, Visiting Fellow, LNNS, NINCDS
J. Dambrosia, Biostatistician, BB
I. Klatzo, Chief, LNNS, NINCDS

COOPERATING UNITS *(if any)*

Hahnemann University, Philadelphia, PA

LAB/BRANCH

Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Cerebrovascular Physiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 0.5	**PROFESSIONAL:** 0.4	**OTHER:** 0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
  ☐ (a1) Minors
  ☐ (a2) Interviews
☐ (b) Human tissues
☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In collaboration with Professor Thomas Devlin of Hahnemann University in Philadelphia, PA an effort was made to evaluation a prostaglandin derivative called PGBx for its protective action against ischemic brain damage. This compound, isolated in the course of studies on stress, was found by earlier workers to protect vitro mitochondrial oxidative phosphorylation from hypoxia. We compared the survival of PGBx treated and untreated adult mongolian gerbils which had been subjected to 15 minutes of bilateral carotid occlusion. Although our first set of studies showed beneficial effects, repeated studies with new supplies of PGBx failed to show the effect even with major variations in dosage and time of dosage. Although this conclusion does not invalidate the metabolic studies which had shown other beneficial effects such as the restoration of oxidative phosphorylation in mitochondria exposed to hypoxia, further studies have been deferred.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02627-02 LNNS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Relationship between electrical impedance and intracranial pressure

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

H.G. Wagner, Chief, Section on Cerebrovascular Physiology, LNNS, NINCDS
P. Ting, Special Expert, LNNS, NINCDS
K. Kito, Visiting Fellow, LNNS, NINCDS
I. Klatzo, Chief, LNNS, NINCDS

COOPERATING UNITS (if any)

None

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SECTION

Section on Cerebrovascular Physiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

AN EVALUATION OF THE ROLE OF INCREASED INTRACRANIAL PRESSURE (ICP) IN PRODUCING BRAIN COMPRESSION AND CHANGES IN CEREBRAL ELECTRICAL IMPEDANCE (CEI)

Our studies showed that focal brain ischemia produced by one hour occlusion of the middle cerebral artery in cats produced a rise in the cerebral electrical impedance of the affected grey matter. The CEI returned approximately to pre-ischemic levels when the occlusion was released. In many of these animals a second later rise in CEI was observed to occur which appeared to be related to an increase in intracranial pressure. To test this hypothesis, brain compression was produced by epidural balloon inflation. When the epidural pressure was increased, the CEI increased as much as 216%. The regional blood flow (rCBF) was lowered but not to ischemic levels. This findings indicated that brain compression produced by edema can itself produce a reduction in extracellular space without necessarily lowering rCBF to critical ischemic levels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02689-01 LNNS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Effects of vasoactive substances in carbohydrate metabolisms in cultured cerebromicrovascular cellular elements

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

M. Spatz, Head, Section on Neurocytobiology, LNNS, NINCDS
B. Wroblewska, Visiting Fellow, LNNS, NINCDS

COOPERATING UNITS (if any)

B.B. Mrsulja, Biochemistry Institute, University of Belgrade Medical School
Belgrade, Yugoslavia

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Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	.1	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The separately cultured smooth muscle and endothelial cells derived from dissociated cerebral microvessels are characterized by high content of glycogen. Norepinephrine induces glycogenolysis while 5-hydroxytryptamine stimulates glycogenesis in both cell types. The endogenenous glucose of the endothelium but not that of the smooth muscle serves as a direct source for the 5-HT enhancement of glycogen formation. Indomethacin, the known inhibitor of cyclooxygenase modulates the glycogen content in the smooth muscle only. These findings strongly suggest that the carbohydrate metabolism of each cell has a distinct control mechanism compatible with the underlying integral microvascular function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02690-01 LNNS

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Effect of forskolin on the growth and differentiation of cultured cells from rat brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

O. Kempski, Guest Worker, LNNS, NINCDS
B. Wroblewska, Visiting Fellow, LNNS, NINCDS
M. Spatz, Head, Section on Neurocytobiology, LNNS, NINCDS

COOPERATING UNITS (if any)

H. Kretschmar Inst. Neuropathology University of California, San Francisco

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SECTION
Section on Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	.8	.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In-vitro studies using cultured brain cells are hampered by the fact that these cells are exposed to high concentration of growth factors from fetal calf serum (FCS) as well as from artifical media. Therefore, they are often in an undifferentiated state and not necessarily representative for a functional cell in-vivo. Possible cAMP involvement in cellular differentiation was examined in cultured cells exposed to either forskolin or dBcAMP.

The evaluation of cellular thymidine incorporation and cAMP production serve to determine the action of these agents. GFAP was used on a measure of glial differentiation.

All three cell types showed a forskolin dose-dependent reduction of thymidine incorporation in the presence of FCS. Maximal inhibition was achieved with 100μM forskolin which reduced thymidine incorporation to levels otherwise found in the absence of FCS (75-95% reduction). 5 day exposure of glial cells to forskolin not only caused striking morphological changes similar to those observed after dBcAMP but also led to an increased expression of GFAP which was demonstrated by immunohistochemistry and ELISA.

These findings stress the importance of cAMP in the regulation of mitotic activity. Moreover, the results suggest that forskolin may serve as a tool to initiate differentiation in brain cells in culture.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02691-01 LNNS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* Ultrastructural observations on the transvascular route of protein removal in vasogenic brain edema

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

I. Klatzo, Chief, LNNS, NINCDS
A.W. Vorbrodt, Research Fellow, Inst. Bas. Research, N.Y.
A.S. Lossinsky, Research Fellow, Inst. Bas. Research, N.Y.
H.M. Wisniewski, Director, Inst. Bas. Research, N.Y.
R. Suzuki, Visiting Fellow, LNNS, NINCDS
T. Yamaguchi, Visiting Fellow, LNNS, NINCDS
H. Masaoka, Visiting Fellow, LNNS, NINCDS

COOPERATING UNITS *(if any)*

Institute for Basic Research in Developmental Disabilities, Staten Island, N.Y.

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Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION

Section on Cerebrovascular Pathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	.8	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our observations suggest that the observed, reverse, vesicular transport of HRP across the endothelial cells of some blood vessels represents one of several possible mechanisms reponsible for the removal of extravasated proteins and of edematous fluid from brain extracellular space. This reverse transport is accompained by a disruption of the surface anionic layer and changed polarity of endothelial cells manifested by the relocation of alkaline phosphatase activity from luminal to abluminal plasmalemma. The newly described mechanism for transvascular route of serum protein removal may play a very significant role in various phases of resolution of vasogenic edema and therefore investigations concerning possible acceleration of such transvascular removal maybe of importance in designing some therapeutic measures in vasogenic brain edema.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02692-01 LNNS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Morphological evaluation of glycon changes in cerebral ischemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

D.E. von Lubitz, Visiting Associate, LNNS, NINCDS
H. Masaoka, Visiting Fellow, LNNS, NINCDS
G. Goping, Biological Laboratory Technician, LNNS, NINCDS
I. Klatzo, Chief, LNNS, NINCDS

COOPERATING UNITS (if any)

None

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SECTION

Section on Cerebrovascular Pathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The morphological changes in histochemically demonstrable glycogen were evaluated following 5 minute bilateral carotid occlusion in gerbils. The first appearance of abnormal increase in glycogen granules was observed in hippocampus after 2 hours following 5 minute ischemia. The accumulation of glycogen in astrocytic cells reached its peak at 6 hours after release of catotid occlusion. This was followed by a striking reduction in glycogen, especially in hippocampus, which was observed at 24 hours. A maximal accumulation of glycogen was conspicuous in Schaffer's collaterals at 48 hr post-ischemic time interval. These observations indicate that periods of perviously demonstrated neuronal hyperactivity are associated with a conspicuous reduction of glycogen, whereas a collapse of neuronal activity corresponds to a conspicuous accumulation of glycogen, mainly in astrocytic cells. The morphological observations on glycogen provide thus an insight into changes in energy metabolism in cerebral ischemia and they contribute to a better understanding of this so clinically and important condition.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02693-01 LNNS

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Features of the blood-brain barrier (BBB) opening to horseradish peroxidase (HRP) at the onset of bicuculline

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

C. Nitsch, Visiting Scientist, LNNS, NINCDS
H. Laursen, Visiting Associate, LNNS, NINCDS
G. Goping, Biologist Laboratory Technician, LNNS, NINCDS
I. Klatzo, Chief, LNNS, NINCDS

COOPERATING UNITS (if any)
None

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INSTITUTE AND LOCATION
NINCDS, NIH, BETHESDA, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	.8	.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Rabbits which had received horseradish peroxidase (HRP) intravenously were subjected to bicuculline-induced generalized seizures of 15 min duration in order to elucidate the mechanism by which macromolecules traverse the blood-brain barrier (BBB) in specific neuroanatomically defined brain areas. As a rule, transendothelial pinocytosis at the level of arterioles was the main route of passage. In addition, tracer-filled pinocytotic vesicles were also observed in the capillaries of the hippocampus, thalamus, whereas in thalamus, hypothalamus and septum, they were present also in the endothelium of venules. Indication of an opening of the interendothelial tight junctions was found in the hypothalamus.

The HRP, which had reached the neuropil due to the seizure-evoked BBB opening, accumulated in the interstitial spaces and penetrated the synaptic cleft region. Uptake of the tracer in vesicular form into presynaptic boutons, presumably excitatory ones, was observed in all brain regions; it was frequent in pallidum, hippocampus and medulla oblongata and very intense in the thalamus. Concomittant uptake in postsynaptic dendrites, was present mostly in the vicinity of boutons. Incorporation into glial processes was rare.

It is concluded, that blood-borne macromolecules traverse the BBB by regionally selective, transmitter-controlled pinocytotic transport and that the neuronal uptake of the foreign protein during the generalized seizures is a least partially dependent on the involvement of synapses of particular brain regions. These studies contribute to the understanding of mechanisms operative in epileptic seizures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02621-02 LNNS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Properties of Glucose 6-Phosphatase in Cerebrovascular Endothelium

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

B.M. Djuricic, International Research Fellow, LNNS, NINCDS
M. Spatz, Head, Section on Neurocytobiology, LNNS, NINCDS

COOPERATING UNITS (if any)

None

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SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.0	.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is part completed, and will be completed at a later time.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02552-04 LNNS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Investigation of extraneuronal catechol synthesizing enzymes in the CNS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

M. Spatz, Head, Section on Neurocytobiology, LNNS, NINCDS

COOPERATING UNITS *(if any)*

Dr. Ikuko Nagatsu, Fujita-Gakuen Univ. School of Med., Toyoake, Aiche, Japan
Dr. Toshiharu Nagatsu, Tokyo Institutes of Technology, Yokhama, Japan

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SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: .8	**PROFESSIONAL:** .1	**OTHER:** .7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our previous immunohistochemical and biochemical studies of cerebral microvessels and cerebrovascular endothelial cultures showed the presence of phenylethanolamine-N-methyltransferase (PNMT) activity in both tissues. Since these extraneuronal tissues contain a catecholamine synthesizing enzyme which is responsible for conversion of norepinephrine to epinephrine, we extended these studies to determine whether vascular PNMT is indeed capable of producing epinephrine from norepinephrine. For this purpose a direct assay of endothelial epinephrine formed from norepinephrine was determined by using high pressure liquid chromatography. These studies, which are still in progress, have shown that the cultured cerebrovascular endothelium (2nd-4th generation) derived from dissociated cerebral microvascular fractions (obtained from rats) are capable of converting norepinephrine to epinephrine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02573-03 LNNS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Changes in water content of brain and BBB in convulsive seizures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

R. Cahn, Visiting Fellow, LNNS, NINCDS
T. Kuroiwa, Visiting Fellow, LNNS, NINCDS
I. Klatzo, Chief, LNNS, NINCDS

COOPERATING UNITS *(if any)*

None

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Section on Cerebrovascular Pathology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 1.3	PROFESSIONAL: .8	OTHER: .5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
  ☐ (a1) Minors
  ☐ (a2) Interviews
☐ (b) Human tissues
☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

CHANGES IN WATER CONTENT OF BRAIN AND BBB IN CONVULSIVE SEIZURES

This project has been completed and the resulting manuscript has been published.

Cahn, R., Kuroiwa, T., Lust, D., Nistch, C. and Klatzo, I.: Changes in cerebrovascular permeability, brain water content and blood osmolarity in short epileptiform seizures. In: Current Problems in Epilepsy. M. Baldy-Molinier, D.H. Ingvar, B.S. Meldrum (eds.). John Libey Eurotext Publ. London-Paris pp. 167-173.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02574-03 LNNS

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
A new histochemical method for the detection of adenylate cyclase with forskolin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
G. Szumanska, Guest Worker, LNNS, NINCDS
M. Spatz, Head, Section on Neurocytobiology, LNNS, NINCDS

COOPERATING UNITS (if any)
None

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SECTION
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INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	.4	.9

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A new histochemical method was developed for the detection of adenylate cyclase (AC) by stimulation of the enzyme activity with forskolin. This method was compared with the technique in which isoproterenol and 5-guanylylimidodiphosphate (GppNp) were used as activators of AC.

The studies revealed that forskolin is not only a suitable activator of AC but is more effective than isoproterenol and GppNp for the demonstration of this enzyme histochemically.

The availability of the method for the detection of AC activity without the necessity of using a hormonal stimulator has a great potential for the evaluation of this enzyme in normal and pathological tissues especially in those cases showing an absence or desensitization of the specific hormonal receptor linkage to AC.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02575-03 LNNS

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The establishment of cerebrovascular smooth muscle culture

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

M. Spatz, Head, Section on Neurocytobiology, LNNS, NINCDS

COOPERATING UNITS *(if any)*

Dr. Ronald F. Dodson, Division of Experimental Pathology, East Tyler Chest Hospital, Tyler, Texas

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INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	**PROFESSIONAL:**	**OTHER:**
1.2	.1	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
  ☐ (a1) Minors
  ☐ (a2) Interviews
☐ (b) Human tissues
☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

THE ESTABLISHMENT OF CEREBROVASCULAR SMOOTH MUSCLE CULTURE

This project has been completed and the resulting manuscript has been published.

> M. Spatz, R.F. Dodson and J. Bembry: Cerebrovascular muscle cultures. I. Isolation, growth and morphological characterization. Brain Research 280: 387-391, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02361-08 LNNS

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Investigations on blood-brain barrier (BBB) permeability.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
M. Spatz, Head, Section on Neurocytobiology, LNNS, NINCDS

COOPERATING UNITS (if any)
Prof. K. G. Go, and Dr. H.J. Hauthoff, Department of Neurosurgery and Pathology, University of Groningen, The Netherlands

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Laboratory of Neuropathology and Neuroanatomical Sciences

SECTION
Section on Neurocytobiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been temporarily discontinued.

Publication:

K.G. Go, H.J. Hauthoff, S.Huitema and M. Spatz
Protein Tracer Permeability of the Blood-Brain Barrier After Transient Cerebral Ischemia In Gerbils.
Recent Progress In the Study and Therapy Of Brain Edema
Edited by K. G. Go and A. Baethmann

PHS 6040 (Rev. 1/84) GPO 914-918

TAB 12 -- LABORATORY OF PHYSIOLOGY -- (LNP)

ANNUAL REPORT

October 1, 1984 through September 30, 1985
Laboratory of Neurophysiology

National Institute of Neurological and
Communicative Disorders and Stroke

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ANNUAL REPORT

October 1, 1984 through September 30, 1985

Laboratory of Neurophysiology, IRP
National Institute of Neurological and
Communicative Disorders and Stroke
Jeffery L. Barker, Chief

During FY 85 the research program emerging in the Laboratory of Neurophysiology noticeably expanded both in terms of direction, personnel and space. There are now some 40 members of the Laboratory engaged in a variety of research projects all of which involve elucidation of specific molecular or cellular properties either under in vitro or in situ conditions. Although the spectrum of research activity already established or in development is quite diverse, all of the experiments are contemporary in technique and strategy, if not innovative. The combination of quantitative structural and functional studies into important cell biolodical properties common to many cellular phenotypes, especially the biology of specific receptors, is unlikely to eclipsed elsewhere. The collective expertise and experience now in the Laboratory should be sufficient to realize some of the long-term goals of our overall research program.

One goal involves quantitative analysis of the ontogenetic development and phylogenetic distribution of specific transmitter receptors. When do certain receptors develop during evolution or arise during development and how are they distributed in functionally distinct nerve, endocrine, immune and cardiac muscle tissues? What is the molecular disposition of specific receptor proteins in membranes and what is their topographic distribution along the surface of specific neuronal phenotypes? A second, related goal involves quantitative analysis of specific receptor functions in phenotypically distinct nerve, endocrine and immune cells using contemporary cell biological assay techniques. How are specific receptors related to ion conductance mechanisms in the membrane or to regulation of cytosolic pCa and pH or to other receptor-coupled membrane and cytoplasmic properties? By focussing on several major classes of transmitters and their receptors we should be able to discover fundamental aspects of receptor structure and function and thereby gain some insight into a "transmitter code", especially as it applies to specific circuits of nerve, endocrine and immune cells.

It has recently become increasingly clear that transmitters and receptors and receptor-regulated changes in membrane excitability are not confined to nerve and muscle tissue, but instead are virtually ubiquitous, being expressed to one degree or another in all types of cell studied thus far. Most of our present projects involve characterization of specific receptor properties and functions exhibited by cellular elements derived from specific regions of the embryonic and adult vertebrate CNS, from clonal and endocrine pituitary tissues, from heart and from human and rodent clonal and primary immune tissues. There are a variety of experimental strategies in the Laboratory either now established or in development. These include 1) high-yield receptor protein purification for adrenergic and cholinergic receptors; 2) analysis of the primary aminoacid sequence and structure of adrenergic and cholinergic receptors; 3) *in vivo* and *in vitro* immunization techniques for the production of monoclonal anti-receptor immunoreagents; 4) quantitative assays of ligand-binding properties of solubilized and native receptors; 5) dissociated primary and clonal cultures of mammalian nerve, endocrine and immune tissues; 6) quantitative electrophysiological and optical recording techniques applied at the single-cell and monosynaptic circuit levels in cultures, in retinal slice and in retinal eyecup preparations; 7) flow cytometric analysis of physiologically important properties in cellular suspensions of embryonic CNS tissue, clonal and primary pituitary tissue and immune cells; 8) flow cytometric isolation of specific cellular phenotypes from nerve, endocrine and immune systems; 9) light- and electron-microscopic resolution of cellular form and subcellular structure in monolayer culture preparations and in normally developed retina; 10) immunohistochemical characterization of transmitter phenotype and surface-receptor expressions in sorted and unsorted monolayers of embryonic CNS cells; 11) quantitative analysis of fluorescence signals expressed by cytoplasmic and membrane determinants in cultured nerve, endocrine and immune cells. This array of biotechnology is considerable and diverse, yet complementary. We should be able to discover when specific receptors become expressed both during embryogenesis and in the course of evolution and what roles they play in the physiological context of chemical signalling. The strength of the Laboratory lies in the opportunity created by the range of innovative strategies. We now have the chance to ask questions regarding the structure and function of specific transmitters and receptors in experimental detail, examining their roles in intercellular communications between specific nerve, muscle, endocrine and immune cells.

Specific scientific advances made during FY 85 and future directions are concisely described in the accompanying project reports (Z01 NS 02019-13; Z01 NS 02330-08; Z01 NS 01659-17; Z01 NS 02631-02; Z01 NS 02670-01; Z01 NS 02671-01; Z01 NS 02672-01). The implementation by Drs. Venter and Fraser and colleagues of innovative receptor-protein-purification protocols coupled with an anti-receptor monoclonal antibody production program is quite important. It expedites collaborative efforts into the structure and function of certain receptors on specific nerve, endocrine and immune cells. Equally significant has been the development of relatively routine flow cytometric analysis and isolation followed by culture of embryonic mammalian motoneurons, mesencephalic dopamine cells, primary prolactinergic pituitary cells and specific effector lymphocytes by Drs. Schaffner, DiPorzio, Dufy, St.John and Mandler. For example, we should soon be able to detect and analyze with flow-cytometry specific receptor distributions in populations of nerve, endocrine and immune cells and then isolate and culture these cells for detailed multi-disciplinary study of receptor function at the single-cell level using the various quantitative electrical, optical and morphological techniques in the Laboratory. Ideally, the dual-colar capability of the cell-sorter will reveal not only receptor expression in subpopulations of phenotypically distinct cells but also simultaneoulsy record certain receptor-coupled functions probed with indicator dyes.

In summary, the Laboratory has developed a strong and varied research program to study the biology of specific receptors expressed in a variety of cellular phenotypes and their roles in physiologically important circuits involving nerve, muscle, endocrine and immune cells. Eventually, we plan to compare data obtained on specific receptor biologies expressed in normal tissues and systems with that found in certain pathophysiological conditions to uncover the possible receptor-related mechanisms involved.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02019-13 LNP

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Electrophysiological Studies on Membrane Excitability

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:.	J.L. Barker	Chief	LNP, IRP, NINCDS
Others:	T.G. Smith	Section Chief	LNP, IRP, NINCDS
	G.D. Lange	Physiologist	LNP, IRP, NINCDS
	B. Dufy	Visiting Scientist	LNP, IRP, NINCDS
	A.B. MacDermott	Staff Fellow	LNP, IRP, NINCDS
	D.G.Owen	Visiting Associate	LNP, IRP, NINCDS
	A.E. Cole	PRAT Fellow	NIGMS
	P. Sheehy	Staff Fellow	LNP, IRP, NINCDS

COOPERATING UNITS (if any) H. Betz (Univ. of Heidelberg, West Germany); H. Lecar (LB, IRP, NINCDS); S. Vicini (Lab Preclinical Studies, IRP, NIMH); S. Smith (Molecular Neuroscience Section, Department of Physiology, Yale University); J.F. MacDoanld (Helen Scott Playfair Neuroscience Unit, Toronto, Canada)

LAB/BRANCH
Laboratory of Neurophysiology, IRP, NINCDS

SECTION
Office of the Chief and Section on Sensory Physiology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
12	11	1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of this research program involves elucidation of the ion permeability mechanisms expressed by primary and clonal cells cultured from the embryonic mammalian CNS and from neuronal-glioma tumor, from endocrine pituitary and from immune tissue. These mechanisms are considered critical in the physiology and diverse functions of the various cellular phenotypes. Specific lines of investigation include projects on embryonic CNS neurons cultured from spinal and supraspinal regions, clonal and primary pituitary cells, and clonal and primary effector lymphocytes. Electrophysiological measurements of excitability are made in membrane patches or in whole-cells, using either low-resistance patch-clamp techniques or high-resistance microelectrodes for recording. The different assay techniques provide complementary data for characterizing the membrane and cytoplasmic mechanisms underlying ion conductances in these cells. Principal observations this year include the following: 1) simultaneous optical and electrical assays of primary CNS neurons and clonal pituitary cells have been developed, allowing correlation of excitable membrane events and $[Ca^{2+}]_i$ changes; 2) electrical recording of membrane capacitance under different secretory conditions correlates well with hormone secretion rates, presumably reflecting increased incorporation of organelle membrane into the cell wall; 3) cell-sorted motoneurons exhibit a full complement of ionic conductances, including one previously uncharacterized, and respond to aminoacids in well-recognizable fashions; 4) the steroid anesthetic alphaxalone amplifies GABA-mediated inhibition in a barbiturate-like way and directly activates inhibitory conductance in a GABAmimetic manner with 100-fold greater potency than barbiturate anesthetics; 5) phosphoinositide metabolism contributes to the development of hormonally-induced K^+ conductance in clonal pituitary cells; 6) skeletal muscle secretes a factor(s) that regulates neuronal excitability in embryonic sensory neurons; and 7) killer-type lymphocytes are electrically excitable.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02330-08 LNP

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Cell Biological Studies of CNS Neurons, Pituitary and Immune Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	J.L. Barker	Chief	LNP, IPR, NINCDS
Others:	M.T. Caserta	Staff Fellow	LNP, IRP, NINCDS
	U. DiPorzio	Visiting Scientist	LNP, IRP, NINCDS
	G.D. Lange	Physiologist	LNP, IRP, NINCDS
	A.P. Mariani	Senior Staff Fellow	LNP, IRP, NINCDS
	P.A. St. John	Senior Staff Fellow	LNP, IRP, NINCDS
	A.E. Schaffner	Senior Staff Fellow	LNP, IRP, NINCDS
	L. Dufy-Barbe	Guest Researcher	LNP, IRP, NINCDS

COOPERATING UNITS (if any) R. Mandler (NIB, IRP, NINCDS); J. Moskal (LCB, NIMH); G. Rougon (LCB, NIMH); H. Mohler (Hoffman-LaRoche, Basel, Switzerland); H. Betz (Univ. of Heidelberg, Heidelberg, Germany); E.A. Grimm (SNB, IRP, NINCDS); P. Henkart (CI, NCD)

LAB/BRANCH
Laboratory of Neurophysiology, IRP, NINCDS

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10	6.5	3.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The immediate aim of this research program is the development of cell biological assays at relatively quantitative levels of resolution for application to cell suspensions and dissociated monolayers of cultured nerve, endocrine and immune cells. The techniques in development include dual-laser fluorescence-activated cell analysis and sorting (FACS), light- and electron-microscopic study of cultured elements using both cytoplasmic and surface-reactive immunologic probes and computer-assisted, quantitative analysis of fluorescence signals from living cells in monolayer culture. Principal observations this year include: 1) a significant and seemingly paradoxical shift in the FACS light-scatter histogram of live cells during embryonic development of the mouse spinal cord but not in histograms of other CNS regions; 2) routine retrograde labelling and FACS isolation of motoneurons and sensory cells followed by long-term culture and morphological and electrophysiological characterization; 3) immunostaining of live prolactin-secreting pituitary cells using anti-prolactin antibody followed by FACS isolation and culture; 4) surface-immunostaining of live embryonic mesencephalic cells followed by FACS analysis, isolation, culture and immunoctyochemical characterization with enrichment for catecholaminergic neurons; 5) immunostaining and morphological characterization of dopamine-containing neurons in the spinal cord both in vitro and in vivo; 6) computer-assisted quantitative analysis of fluorescence signals in single nerve, endocrine and immune cells; 7) FACS analysis of effector lymphocytes and their conjugation with tumor target cells. The techniques and protocols used in these projects represent complementary ways of assaying functionally important properties in different cellular phenotypes in a quantitative manner at the single-cell level of experimental resolution.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01659-17-LNP

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Synaptic Contacts of Retinal Neurons

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

A. Lasansky Chief, Section on Cell Biology LNP, IRP, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Neurophysiology, IRP, NINCDS

SECTION
Section on Cell Biology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Simultaneous recordings from cone-horizontal cell pairs have been performed by means of intracellular microelectrodes in slices of salamander retina. Cone responses following electrical stimulation of the horizontal cell have been observed in only one instance, possibly because the slicing procedure disrupts the horizontal cell network.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02631-02 LNP

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function in Retinal Neurons

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. :	Ralph Nelson	Physiologist	LNP, NINCDS
Others:	Helga Kolb	Prof., Dept. Physiol.	University of Utah
	Andrew P. Mariani	Senior Staff Fellow	LNP, NINCDS
	Renata Pflug	Visiting Associate	LNP, NINCDS
	Michael Freed	Guest Researcher	LNP, NINCDS
	Jan Nora Moura de Melo	Guest Researcher	LNP, NINCDS
	Kieth Purpura	Guest Researcher	LNP, NINCDS

COOPERATING UNITS (if any)

Department of Physiology, University of Utah, Salt Lake City, Utah (H. Kolb)

LAB/BRANCH

Laboratory of Neurophysiology, IRP, NINCDS

SECTION

Neural Circuitry Unit

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to elucidate the anatomical, electrophysiological and neurochemical properties of neurons in mammalian retinas and to infer the interactions and interconnections which comprise retinal neural circuits, and so to obtain further insights into retinal function in normal and diseased states.

Color vision in cats is controversial, nonetheless studies of the spectral sensitivities of cat horizontal cells have revealed three cone types: a blue type with a peak sensitivity at 440 nm, a green type peaking at approximately 520 nm, and a red type maximally sensitive at 560 nm. Interactions among the cones have also been observed with suggestions of blue inhibiting green, and green inhibiting red. Some cat horizontal cells are shown to be spectrally tetrachromatic with input from these three cone types as well as rods. The contributions of the two shorter wavelength peaking cones become especially prominent on dim red backgrounds. Although different horizontal cells show partial selectivity for cones, this appears unrelated to the classical anatomical divisions between the axonless A-type cells and the axon bearing B-type cells. Only the red cones were found to be dynamically rapid enough to follow 10 Hz flicker, leading to a convenient test to separate red from blue and green cone signals. Surprisingly, one rod-connected horizontal cell axon terminal, although exhibiting rod-dominated responses in the dark adapted state, showed a dominant blue cone selectivity with red backgrounds. Results suggest that interreceptor contacts between cones may function to intermix signals from different chromatic types of cone at the receptor level.

[This project was transferred from the Laboratory of Neurochemistry under which the staff support man-years were performed.]

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02670-01 LNP

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Evolution of Neurotransmitter Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.C. Venter	Section Chief	LNP, IRP, NINCDS
Others:	C.M. Fraser	Research Physiologist	LNP, IRP, NINCDS
	S. Fracek	Staff Fellow	LNP, IRP, NINCDS
	S.M. Shreeve	Fogarty Associate	LNP, IRP, NINCDS
	A. Kerlavage	Sen. Staff Fellow	LNP, IRP, NINCDS
	D. Robinson	Guest Researcher	LNP, IRP, NINCDS
	J. Earle	Guest Researcher	LNP, IRP, NINCDS
	L. Cohen	Biologist (Tech)	LNP, IRP, NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Neurophysiology, IRP, NINCDS

SECTION
Section on Receptor Biochemistry

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	2.1	0.7

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to examine the structural and functional evolution of neurotransmitter receptor proteins. Using muscarinic cholinergic receptors as the initial model system we have isolated and characterized the muscarinic acetylcholine receptor from the brains and hearts of a wide variety of species. Brain and heart tissue have been obtained from all vertebrate classes including mammals, birds, reptiles, amphibians, and fish. In addition, lower species including insects (drosophila) have provided receptors. The studies underway indicate that of all of the studied parameters; SDS gel molecular weight, isoelectric points, monoclonal antibody cross reactivity, agonist affinity, antagonist affinity, stereospecificity, GTP shifts, and receptor density, only the last parameter, receptor density, appears to have changed by increasing gradually over 900 million years of evolution.

Muscarinic receptors are being purified from human brain, rat brain and heart, pig heart and shark brain and heart in order to compare in detail primary structures. The evolutionary relationship between muscarinic cholinergic receptors and other neurotransmitter receptors is also being studied. Protein sequencing and gene probes will be used to follow the evolution of adrenergic, cholinergic, dopaminergic, serotonergic, GABAergic and opiate receptor proteins.

Neurotransmitter biosynthetic/degradative enzymes, e.g. tyrosine hydroxylase, CAT, acetylcholinesterase will be examined with monoclonal antibodies and primary sequence data for any evolutionary/structural relationship with the neurotransmitter receptor proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02671-01 LNP

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Immunological Characterization and Localization of Neurotransmitter Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	C.M. Fraser	Research Physiologist	LNP, IRP, NINCDS
Others:	J.C. Venter	Section Chief	LNP, IRP, NINCDS
	S.M. Shreeve	Fogarty Associate	LNP, IRP, NINCDS
	J. Earle	Guest Researcher	LNP, IRP, NINCDS
	D. Robinson	Guest Researcher	LNP, IRP, NINCDS
	L. Cohen	Biologist (Tech)	LNP, IRP, NINCDS
	P. Lee	Biologist (Tech)	LNP, IRP, NINCDS
	D. Cosenza-Murphy	Biologist (Tech)	LNP, IRP, NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Neurophysiology, IRP, NINCDS

SECTION
Section on Receptor Biochemistry

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.3	1.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aims of this research are to develop a more extensive library of monoclonal antibodies to major classes of neurotransmitter receptors including muscarinic cholinergic, alpha and beta-adrenergic, dopaminergic, serotonergic, GABAergic and opiates; and to utilize these reagents in studies of receptor structure and function, the degree of molecular homology between receptor subclasses, the localization of neurotransmitter receptors on cell membranes and the evolution of receptor subclasses.

Principal observations to date include the following: 1) monoclonal antibodies raised against each major class of neurotransmitter receptor recognize the same receptor in a number of different tissues and species suggesting considerable conservation of receptor structure throughout evolution; 2) certain monoclonal antibodies raised against muscarinic cholinergic receptors recognize alpha$_1$-adrenergic receptors and vice versa suggesting structural homology between these pharmacologically distinct receptor sub-types; 3) monoclonal antibodies raised against alpha$_1$-adrenergic receptors recognize alpha$_2$-adrenergic receptors and this information taken together with pharmacological and biochemical data suggests that alpha$_1$- and alpha$_2$-adrenergic receptors may be closely related "isoreceptors"; 4) monoclonal antibodies to muscarinic cholinergic receptors have been utilized to fluorescently label receptors on the surface of cultured cells demonstrating the potential usefulness of these reagents in studies of receptor localization and distribution in various species and tissues as well as in studies of receptor processing and turnover.

Purified receptor antigen are also being utilized to further characterize autoimmune-receptor related disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02672-01 LNP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurotransmitter Receptor Purification and Structure

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.C. Venter	Section Chief	LNP, IRP, NINCDS
Others:	C.M. Fraser	Research Physiologist	LNP, IRP, NINCDS
	S.M. Shreeve	Fogarty Associate	LNP, IRP, NINCDS
	S. Fracek	Staff Fellow	LNP, IRP, NINCDS
	A. Kerlavage	Sen. Staff Fellow	LNP, IRP, NINCDS
	D. Robinson	Guest Researcher	LNP, IRP, NINCDS
	P. Lee	Biologist (Tech)	LNP, IRP, NINCDS
	J. Earle	Guest Researcher	LNP, IRP, NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neurophysiology, IRP, NINCDS

SECTION

Section on Receptor Biochemistry

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.8	2.9	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Neurotransmitter receptors; adrenergic (beta$_1$, beta$_2$, alpha$_1$ and alpha$_2$), cholinergic (muscarinic and nicotinic), dopaminergic, and serotonergic receptors are being isolated and purified in order to understand the molecular basis of receptor function and neuronal communication. Specific projects are underway to provide precise structural information on each of the above receptor proteins. Structural data being obtained include primary sequence data, proteolytic digest maps, topology information and structure-function data, e.g. neurotransmitter binding site localization, sugar localization, membrane domain and effector coupling protein recognition domains. Our data have demonstrated that structural similarities exist amongst non-pharmacologically related neurotransmitter receptors (muscarinic, cholinergic and alpha adrenergic) and that these neurotransmitter receptors mediate cellular modulation via protein conformational changes initiated by neurotransmitter binding to the binding site in the extracellular protein domain. Receptor coupling is mediated by the cytoplasmic "tail" of the receptors which appears to be the effector protein (GTP-regulatory protein) recognition portion of the receptor.

Protein preparative procedures have been established which include various HPLC steps, ligand affinity chromatography, monoclonal antibody affinity chromatography, preparative SDS-gel electrophoresis, lectin affinity chromatography, ion exchange chromatography and column isoelectric focusing. The establishment of these purification protocols are now permitting simultaneous detailed structural comparisons of all adrenergic and cholinergic receptor proteins. Functional receptor proteins isolated by affinity chromatography and HPLC are being reconstituted into phospholipid vesicles together with purified GTP-regulatory proteins (G_i and G_o) to study molecular events involved in the control of cellular events by receptor proteins.

TAB 13 -- BIOMETRY AND FIELD STUDIES BRANCH -- (BFSB)

ANNUAL REPORT
October 1, 1984 through September 30, 1985
Biometry and Field Studies Branch

Intramural Research Program
National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT
for period October 1, 1984 through September 30, 1985

Biometry and Field Studies Branch

Intramural Research Program
National Institute of Neurological and Communicative Disorders and Stroke

Jonas H. Ellenberg, Ph.D., Acting Chief

The Biometry and Field Studies Branch (BFSB) supports a program in biostatistics and computer science to advance the mission of NINCDS in the areas of neurology and communicative disorders. The Branch participates in a wide range of intramural and extramural collaborative projects, including large-and small-scale observational studies, clinical trials and laboratory studies. These collaborative studies are conducted through both direct staff research and through research and development contracts. In addition to collaborative work, the Branch has an important research component in statistical methodology.

I. COLLABORATION WITH THE INTRAMURAL AND EXTRAMURAL RESEARCH PROGRAMS, NINCDS

Our current collaborative research program has developed primarily in response to requests for collaboration from Intramural and Extramural scientists at NINCDS. Typically, BFSB assumes responsibility for the statistical design, data management and statistical analysis aspects of the projects, with the Program providing the project initiatives, subject matter expertise and overall project leadership.

An active area of collaborative research with the Stroke and Trauma Program (STP) involves the Cerebrovascular Clinical Research Master Agreement. Three studies of interventional stroke therapies are in progress. A dose escalation/pilot study of hypervolemic hemodilution (Dextran-40) for the treatment of cerebral ischemic stroke-in-evolution has accrued twenty-two patients and will continue patient accrual and follow-up through 1985. The planning and design for a study of nicardipine, a calcium channel blocker, for the prevention of vasospasm following subarachnoid hemorrhagic strokes was completed and patient accrual began in July, 1985. A pilot study of high dose naloxone (4 g/m^2) for the treatment of acute cerebral ischemia is continuing. This study is based on the results of a prior dose-escalation study that established the maximum-tolerated dose without toxicity and adverse effects. In FY '86, two additional studies will be initiated under the STP Master Agreement and BFSB will be responsible for the statistical components of these studies.

In collaboration with the Convulsive, Developmental and Neuromuscular Disorders Program (CDNDP), the clinical trial of behavioral and cognitive side effects of phenobarbital used for the prevention of febrile seizure recurrence is into the final year of patient accrual. Over 170 children have been randomized to treatment and 100 normal controls have been recruited for the study. Follow-up of patients will continue for a minimum of two and one-half years. BFSB has acted in the capacity of a comprehensive operations center for this study, which has required intensive monitoring of patient accrual, data quality control and data analyses for the trial's monitoring committee. The study has made use of the Medical Studies Database System (MSDS) developed by BFSB staff.

A second collaborative effort with the CDNDP is a population-based study of the prognostic value of the EEG for subsequent seizure activity in children who experienced a febrile seizure. The cooperating medical center is the Pediatric Clinic in Skopje, Yugoslavia. The recruitment of new cases ended in December, 1984, and periodic follow-up (including repeat EEG's and neurologic and physical examinations) will continue through December, 1987. The study includes 400 children with a normal or non-specific abnormal EEG following a first febrile seizure, as well as about 150 children with a specific abnormal EEG following a seizure. The major focal points of the study are recurrent febrile and afebrile seizures and their relationship to the initial EEG, subsequent EEG changes, and the influence of other medical and demographic factors.

BFSB has worked with CDNDP on the survey of medical practice in the management of children with febrile seizures. This survey was based on a probability sample of approximately 10,000 physicians on the American Medical Association list of child neurologists, neurologists, pediatricians, family practitioners, and general practitioners. The primary information obtained from the survey concerned method of treatment, factors that determine treatment or consultation, reasons for chronic treatment, and preferred medications. The data have been analyzed and a manuscript reporting the findings has been completed.

BFSB is participating with the Communicative Disorders Program on a study of factors associated with the acquisition of reading and writing skills by the deaf. Information on educational and family background and on language skills will be examined to determine which, if any, of the variables are associated with reading and writing skills. Planning and study designs have been completed, data collection forms are being tested, and accrual of subjects will begin in FY '86.

The BFSB has worked with several branches and laboratories in the Intramural Research Program during the past year. The Survey of Major Neurological Disorders in Copiah County, a joint project with the Neuroepidemiology Branch, is nearing completion. Reports on stroke and epilepsy have been prepared and submitted for publication. Work on psychomotor delay and a summary and overview paper of the disorders covered by the survey are in preparation. The successful completion of this project, with its important findings with regard to racial differences for major neurological disorders, is a major joint accomplishment of the two Branches. The methodology developed for this project provides an effective model for other studies attempting to obtain prevalence rates of neurological disorders in geographically defined populations.

BFSB is working with the Medical Neurology Branch on a clinical trial of W-544, a new drug for the treatment of intractable partial seizures. The planning and statistical design of the study have been accomplished and patient recruitment is now underway. The study uses a randomized, double-blind, three-period-crossover design, allowing unbiased estimation of treatment effect even in the presence of period-to-period carryover effect. The duration of the study will be approximately two and one-half years with each patient hospitalized for about three months.

Other collaborative studies in IRP include: an evaluation of stroke treatment in a gerbil model (LNNS and LN); a statistical study of space-time clustering of Creutzfeld-Jakob disease and an examination of the relation of polio viruses to Alzheimer's disease (LCNSS); a study of the relationship of viral antibodies to MS (ID); and a study of dopaminergic modulation of DES-induced proliferation of the anterior pituitary gland of the Fisher 344 rat (ET) and the effect of calcium channel modifiers on prolactin release (ET).

The analysis of data from the Collaborative Perinatal Project (NCPP) has continued throughout this year in collaboration with CDNDP. Work in the major areas of cerebral palsy and convulsive disorders will be completed by the end of the 1985 calendar year. A paper on univariate risk factors for cerebral palsy, one on antecedents of low and very low birthweight and their relationship to cerebral palsy and a manuscript that addresses the issue of whether seizures in children are associated with intellectual deterioration, have been completed this year. Work will continue on the maternal infection studies, with a paper on the relationship of toxoplasmosis during pregnancy and childhood outcome completed this year. A study investigating the association of migraine with other diseases and the familial relationship of migraine in mothers and morbidity in their children is also in progress.

II. COLLABORATION WITH SCIENTISTS OUTSIDE OF NIH

Collaborative projects with scientists outside of NIH constitute another important area of activity. One of these projects is a cooperative investigation of the clinical characteristics and outcomes of head injured patients in two differenct regions of the world, designed by the Departments of Neurosurgery at the University of Virginia and the All India Institute of Medical Science (AIIMS), New Delhi. Another project, in collaboration with the Department of Obstetrics at George Washington University, concerns the relationship between premature rupture of membranes and neurologic morbidity in the child.

The BFSB worked with the Department of Psychiatry at Johns Hopkins University to complete a comprehensive neuropsychiatric evaluation to identify the number of cases of dementia in a probability sample of the eastern Baltimore population. Data analysis is in process, with the aim of exploring which components of the comprehensive dementia workup provide the highest specificity and sensitivity when used as a dementia screening test.

In collaboration with the National Institute on Aging, data collected by the NHANES I and the NHANES follow-up surveys are being used to determine the course and prognosis associated with visual and hearing impairment in elderly subjects. The study aim is to identify cohorts of patients at increased risk for deterioration of their functional level.

III. CLINICAL DATA BANKS

BFSB continues its responsibility for the management and operation of the Stroke and Traumatic Coma Data Banks. These data banks provide a resource for addressing research questions on the characteristics, clinical course, and outcome of hospitalized stroke and coma patients. The data bank approach involves the collection of clinical and laboratory data at several clinical centers using a common set of data forms. Each data bank is a collaborative effort between BFSB, which is the coordinating center, and four hospital centers.

During this past year, the several functions of the Data Bank Maintenance Center (DBMC) at Beth Israel Hospital, whose contract was terminated at the end of September, 1984, have been transferred to BFSB, to a contractor (RLR Associates) and to the clinical data bank centers. BFSB has designed and implemented a new data base system at NIH (DCRT) to replace that vacated by Beth Israel. In addition, a patient tracking system to monitor patient accrual and completed follow-up testing was designed and implemented. RLR Associates' workscope has been expanded from design, maintenance, and telecommunications aspects of the "front-end" micro-computer system to include transfer of data from the micro-computer to DCRT, and programming support for the building and updating of the data bases at DCRT. The staff at the clinical centers rely upon center staff for retrieval of data. DCRT is now the data repository for each of the data banks, and data are now flowing directly to DCRT via the micro-computer "front-end" system.

Stroke Data Bank

Data collection for the main phase of the Stroke Data Bank began in FY '83. By the end of FY '85 over 1,500 patients will have been entered. Patients will be followed until the end of the study in June, 1987.

Studies to measure the reliability and validity of portions of the Stroke Data Bank protocol were completed during this year. In a study of agreement in diagnosis, each of several neurologists received completed data bank forms describing the clinical course and work-up for 17 stroke patients, and CT and angiography slides. They were then asked to complete the data bank diagnosis and CT scan forms. Substantial agreement for the detailed classification of stroke using the Stroke Data Bank definitions was found among the six neurologists. A study on the agreement of Data Bank neurologists in the interpretation of CT scans is currently in the analysis phase. A study of reliability and validity of the Center of Epidemiologic Studies Depression Symptoms Scale (CES-D) has also been completed and a manuscript describing the results has been submitted for publication. It was demonstrated that this brief questionnaire was a valid and reliable tool for assessing depression in nonaphasic stroke patients.

Traumatic Coma Data Bank

Data collection for the main phase Traumatic Coma Data Bank began in FY '84. By the end of FY '85 over 400 severely head-injured patients will have been enrolled in the study.

A study to measure the reliability of measures of ventricular brain rates and other CT scan findings has been designed and is scheduled to begin data collection in FY '85. This study is also intended to provide estimates of the accuracy of hand drawn and calculated ventricular brain rate measures compared to machine calculated measures. This is needed because only two of the four coma centers have the equipment to generate ventricular brain rates directly.

The use of the Abbreviated Injury Scale for classifying multiple injuries to coma victims was studied. An expert on the Abbreviated Injury Scale independently coded injuries on over 50 coma patients. In comparing the expert's codings with those of data bank nurses, significant biases were detected. A revised collection form and protocol was written to clarify the coding distinctions and reduce the likelihood of error.

BFSB computer scientists continue to develop new techniques for automated data collection. For example, a system has been developed and is currently being tested, which will enable physicians at the clinical centers to transmit patient intracranial pressure (ICP) measures directly from the patient's bedside to the data bank computer system. This will allow for a linkage of ICP data with other measures of status during the acute care of the severely head injured patient.

Other Activities

BFSB is assisting in a joint study between the Traumatic Coma Data Bank Centers and the Epidemiology Program at the University of Sydney, Australia, using both pilot and main phase Traumatic Coma Data Bank data. Consultation with other NIH Institutes and with academic medical centers is continuing on the design and implementation of data banks in other chronic diseases, such as multiple sclerosis and liver disease, and also on a collaborative clinical study of patients with open head injury.

IV. SURVEYS AND DEMOGRAPHIC STUDIES

With the retirement of the Chief of the Section on Surveys and Demographic Studies and the completion of several major national surveys, BFSB will no longer devote major resources to this area. The current needs of the Institute for morbidity and mortality statistics, as well as the other analytic information arising out of these types of studies have been substantially met.

V. METHODOLOGICAL RESEARCH IN STATISTICS

BFSB statisticians continue to develop new statistical methodology and derive innovative modifications of statistical techniques to meet the needs of the Institute for the design of experiments and field studies, analysis of data, and statistical modeling of biological processes and phenomena. Most of the statistical problems addressed arise from collaborative studies with the Intramural and the Extramural Programs. In general, there are two objectives associated with these various statistical activities of BFSB. The primary objective is the development and improvement of statistical methodology to meet the needs of the

Institute. The secondary objective is to make contributions to the development of statistical methodology which may be more generally useful in neurological and other medical research.

A partial listing of new statistical applications to neurological problems includes: modified metrics for space-time clustering of rare disease applied to a population in a defined geographic area; an autoregressive model of patient response for a k-period-2-treatment crossover drug trial that accounts for both treatment residual effects and random effects for the individual patient; use of incomplete observations in statistical models derived by stepwise variable selection procedures; statistical analysis methods for multiple sclerosis disability data such as sojourn time between exacerbation and nonlinear ordinal disability categories; methods for adjustment of the effect of concomitant variables in categorical data analysis; and sampling strategies for rare neurological disorders.

Theoretical statistical work included: the effect of misclassification of exposure variables on case-control studies; the non-null distribution of statistics that measure spatial clustering; the effect of randomization on heterogeneous populations; modeling Markov transition probabilities for a two-state chain with the incorporation of covariate information; new hypothesis testing procedures in the presence of inequality constraints; a demonstration of the adequacy of the diffusion process as an approximation of a binomial random walk for estimating absorption probabilities; the development of a quantitative measure of bias of the Kaplan-Meier statistic as a function of the dependence of the censoring process; and nonparametric methods for quadratic alternatives.

This past year an active NIH-wide statistical seminar series has been initiated by the Branch. General topics on statistical methods have been presented by notable academic statisticians on topics such as the analysis of survival data, sequential methods of testing time to event data, analysis of repeated measurements, and stochastic modeling in neurobiology (with special emphasis on neural spike train models).

In summary, BFSB is involved in a strong program of collaborative research. Our collaboration extends throughout the Institute on projects with both Intramural and Extramural scientists, and also involves collaboration with scientists outside of NINCDS. The broad scope of our research activity ranges from small, one-on-one collaboration with intramural scientists, to the conduct of large-scale, multicenter clinical data banks. BFSB also makes an important and continuing contribution to statistical methodology applicable to neurological research.

CONTRACT NARRATIVE
Biometry and Field Studies Branch, IRP, NINCDS
Fiscal Year 1985

1. UNIV. OF MARYLAND (N01-NS-2-2302)

2. NEUROLOGICAL INSTITUTE - COLUMBIA UNIV. (N01-NS-5-2384)

3. BOSTON UNIV. (N01-NS-2-2398)

4. MICHAEL REESE HOSPITAL & MEDICAL CENTER (N01-NS-2-2399)

Title: Full Phase Stroke Data Bank

Date Contracts Initiated: July 1, 1982

Contractors' Principal Investigators:
1. Dr. Thomas Price
2. Dr. Jay Mohr
3. Dr. Philip Wolf
4. Dr. Louis Caplan/Dr. Daniel Hier

Current Annual Levels FY'85:
1. $238,000 (Estimated)
2. $347,000 (Estimated)
3. $239,000 (Estimated)
4. $268,000 (Estimated)

Objectives: The primary objective of this project is to implement the full phase of the stroke data bank study, which will collect uniform longitudinal data on stroke patients and will provide a clinical research resource for clinical studies of patients with stroke. This is a collaborative project which involves four clinical centers for the collection of data, and BFSB, which has responsibility for data storage and data analysis.

Methods Employed: The Steering Committee, composed of the Principal Investigators and BFSB personnel, met during the first year of this project, outlined research objectives, and developed forms and data collection protocols. Initial studies in the main phase, during the current year, have focused on research methodology and have included assessment of similarities and differences in administering and recording neurological examinations among the centers.

Significance to the NINCDS Program and Biomedical Research: The Full Phase Stroke Data Bank will provide a resource of high quality data on the clinical course of stroke. The project serves as a prototype for national data bank networks for other neurological disorders.

Proposed Course of the Project: This is the beginning of the third year of a five-year project. The initial course has included determination of research questions to be investigated and design of forms to collect the data. Data collection began in July, 1983, and, as of May, 1985, information on over 1,300

(N01-NS-2-2302)
(N01-NS-5-2384)
(N01-NS-2-2398)
(N01-NS-2-2399)

patients had been collected. Data exploration and analysis is continuing. In addition, the Stroke Data Bank has been invited to publish a Supplement to Stroke describing the data bank and its methodology, which will include the forms that have been developed for data collection. Work on this is proceeding.

Publications:

Shinar, D., Gross, C.R., Mohr, J.P., Caplan, L.R., Price, T.R., Wolf, P.A., Hier, D.B., Kase, C.S., Fishman, I.G., Wolf, C.L., and Kunitz, S.C.; Interobserver variability in the assessment of neurologic history and examination in the stroke data bank. Arch. Neurol. 42(6): 557-565, 1985.

CONTRACT NARRATIVE
Biometry and Field Studies Branch, IRP, NINCDS
Fiscal Year 1985

1. UNIV. OF TEXAS-GALVESTON AND BAYLOR UNIV. MEDICAL COLLEGE (N01-NS-3-2339)
2. UNIV. OF CAL. IN SAN DIEGO (N01-NS-3-2340)
3. MEDICAL COLLEGE OF VIRGINIA (N01-NS-3-2341)
4. UNIV. OF VIRGINIA (N01-NS-3-2342)

Title: Full Phase Traumatic Coma Data Bank

Date Contracts Initiated:
1. April 15, 1983
2. April 15, 1983
3. June 1, 1983
4. July 1, 1983

Contractors' Principal Investigators
1. Dr. Howard Eisenberg
2. Dr. Lawrence Marshall
3. Dr. Donald Becker
4. Dr. John Jane

Current Annual Level FY'85
1. $242,000 (estimated)
2. $256,000 (estimated)
3. $215,000 (estimated)
4. $206,000 (estimated)

Objectives: The primary objective of this project is to implement a full phase computerized interactive data bank which will provide a research resource for numerous ongoing clinical investigations of patients with head injury. This is a collaborative project, which involves BFSB as the data base maintenance center using the NIH computer to store and manipulate the data, and involves four clinical centers for the collection of data, and staff at BFSB, who have the responsibility for data oversight and analysis.

Methods Employed: The Steering Committee, composed of the Principal Investigators and BFSB personnel, met during the initial year of this project and outlined the research objectives, developed forms and a new data collection protocol based on the findings of the pilot Traumatic Coma Data Bank. Data collection began in January, 1984. In the first year of data collection, over 200 patients were enrolled. A major subproject has been initiated. This is a study of how to optimally monitor, record, sample, synthesize, and report intracranial pressure (ICP) data.

Significance to the NINCDS Program and Biomedical Research: Longitudinal data on head-injured victims will be collected at four centers, using uniform definitions and procedures. This information will provide a large body of comparable data for clinical research on the factors influencing survival and quality of life following severe head injury. The number of therapies and monitoring devices commonly utilized during the acute phase of managing traumatic coma necessitates a highly organized data handling capacity, and the data bank will serve as an efficient mechanism for collecting, storing and retrieving this information as well as follow-up data.

(NO1-NS-3-2339)
(NO1-NS-3-2340)
(NO1-NS-3-2341)
(NO1-NS-3-2342)

Proposed Course of the Project: This is the third year of a five-year project. Data collection is continuing and analysis will begin as soon as sufficient data becomes available for specific research questions.

Publications:

1. Toutant, S.M., Klauber, M.R., Marshall, L.F., Toole, B.M., Bowers, S.A., Seelig, J.M., and Varnell, J.B.: Absent or compressed basal cisterns on first CT Scan: Ominous predictors of outcome in severe head injury. J. Neurosurg. 61: 691-694, 1984.

2. Klauber, M.R., Toutant, S.M. and Marshall, L.F.; A model for predicting delayed intracranial hypertension following severe head injury. J. Neurosurg. 61: 695-699, 1984.

3. Seelig, J.M., Marshall, L.F., Toutant, S.M., Toole, B.M., Klauber, M.R., Bowers, S.A., and Varnell, J.B.: Traumatic acute epidural hematoma: Unrecognized high lethality in comatose patients. Neurosurg. 15(5), 617-620, 1984.

CONTRACT NARRATIVE
Biometry and Field Studies Branch, IRP, NINCDS
Fiscal Year 1985

RLR & ASSOCIATES, INC., Fairfax, Virginia (N01-NS-2-2315)

Title: Front-end Microprocessor Support for Data Bank Projects

Date Contract Initiated: June 30, 1982

Contractor's Project Director: Robert L. Rush

Current Annual Level FY '85: $144,000

Objectives: To provide the Stroke and Traumatic Coma Data Bank projects (N01-NS-2-2302, 2398-9, N01-NS-5-2384, N01-NS-3-2339-42) with a front-end software package for cost-effective interactive data entry, updating, editing and nighttime transmission to a host computer, to provide support to the Data Bank Maintenance Center (the Computer Applications Section, BFSB), to provide efficient storage, retrieval and management of the collected data, and to design and implement software additions, enhancements, and maintenance of the existing system.

Major Findings: The contractor provided considerable and significant support during the transfer of the DBMC from the canceled contractor, Beth Israel (N01-NS-2-2308), to the Computer Applications Section, BFSB.

Significance to the NINCDS Program and Biomedical Research: The front-end is an integral part of the Stroke and Traumatic Coma Data Bank Projects, which were established to collect and maintain medical data for both patient management and clinical research. Data storage, retrieval and management of the collected clinical data is essential to fulfill the objectives of the Data Bank Projects.

Proposed Course of the Project: This project will continue throughout the Main Phase Stroke and Traumatic Coma Projects.

Publications: None

CONTRACT NARRATIVE
Biometry and Field Studies Branch, IRP, NINCDS
Fiscal Year 1985

BETH ISRAEL HOSPITAL (N01-NS-2-2308)
BOSTON, MASSACHUSETTS

Title: Data Bank Maintenance Center for Data Bank Network Projects in Stroke and Traumatic Coma

Date Contract Initiated: September 30, 1982

Contractor's Project Director: Dr. Howard Bleich

Current Annual Level FY'85: -0-

Objectives: Beth Israel Hospital was the Data Bank Maintenance Center (DBMC) for the Stroke and Traumatic Coma Data Banks. This contract was terminated at the end of FY '84, at the convenience of the government. The functions of the DBMC have been taken over by a combination of efforts. The Computer Applications Section, BFSB, has assumed management and coordination responsibilities. The responsibility for software development has been incorporated through an expansion of the workscope, into the contract with RLR Associates (N01-NS-2-2315). Retrieval support has been decentralized to the clinical centers, and data are now housed at NIH (DCRT).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02637-02 BFSB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Stroke and Trauma Program Phase I-II Studies of Stroke Therapies*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James M. Dambrosia	Chief, Mathematical Statistics Section	BFSB, IRP, NINCDS
Others:	Richard Raubertas	Mathematical Statistician	BFSB, IRP, NINCDS
	Karlin Richardson	Programmer	BFSB, IRP, NINCDS

COOPERATING UNITS *(if any)*
Stroke and Trauma Program, NINCDS; University of Pittsburgh; University of S. Alabama; University of Iowa; University of Cincinnati; New York University Medical Center

LAB/BRANCH
Biometry and Field Studies Branch

SECTION
Mathematical Statistics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	0.6	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project includes all statistical aspects of design, planning, data coordination and management, and analysis for studies of interventional therapies initiated by task orders issued under the aegis of the STP Master Agreement. Currently three studies, each with two clinical centers, are in various stages of operation. A pilot study of treatment of acute cerebral ischemia with large doses of Naloxone, a pilot study of the benefits of hypervolemic hemodilution (DEXTRAN-40) for the treatment of stroke-in-evolution, and a dose-escalation Phase II study of Nicardipine, a calcium channel blocker, for the prevention of vasospasm following subarachnoid hemorrhage are ongoing. A dose-escalation study of Naloxone was completed prior to the initiation of the pilot study. No major dose-related side effects occurred and a maximal reasonably tolerated dose was determined to be 160 mg/m^2 loading with total dose after infusion of 4 g/m^2.

Two additional studies of stroke treatment will be initiated in FY'86 under the Master Agreement and BFSB will have responsibility for the statistical aspects of these projects.

*[This project supports the Stroke and Trauma Program contract entitled: Cerebrovascular Clinical Research Master Agreement. The Project Officer is Dr. John Marler.]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02444-06 BFSB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Statistical Coordinating Center for the Phenobarbital Clinical Study*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Young Jack Lee	Mathematical Statistician	BFSB, IRP, NINCDS
Others:	Jonas H. Ellenberg	Deputy Chief	BFSB, IRP, NINCDS
	Karin B. Nelson	Chief, Cerebral Palsy and Other Motor Disorders Section	DNB, CDNDP, NINCDS
	Deborah G. Hirtz	Pediatric Neurologist	DNB, CDNDP, NINCDS
	Karlin Richardson	Programmer	BFSB, IRP, NINCDS
	Kenneth Elsner	Systems Analyst	BFSB, IRP, NINCDS
	Dolores Jones	Computer Assistant	BFSB, IRP, NINCDS

COOPERATING UNITS (if any)
Cerebral Palsy and Other Motor Disorders Section, DNB, CDNDP, NINCDS;
University of Washington

LAB/BRANCH
Biometry and Field Studies Branch

SECTION
Mathematical Statistics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.6	0.6	2.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During this fiscal year, one interim analysis of the phenobarbital clinical study data were performed for evaluation by the Performance and Safety Monitoring Committee. The system programs for monitoring patient status and data tracking have been modified to accommodate the change of the contract from a best-effort contract to a fee-for-deliverables contract. Accumulating data are continually being analyzed for consistency and for deviations from protocol using the BFSB H-P clinical trials computer management system. All edited data are transferred from the H-P to the DCRT, NIH computer where data analysis files are created and maintained.

*[This study supports the DNB/CDNDP/NINCDS contract study entitled: "Behavioral and cognitive side effects of phenobarbital used for prevention of febrile seizure recurrence." The project officer is Dr. Karin B. Nelson, DNB, CDNDP, NINCDS, and the contractor of the study is the University of Washington.]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02502-04 BFSB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Medical Studies Database System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Karlin Richardson	Systems Programmer	BFSB, IRP, NINCDS
Others:	Sylvia Edelstein	Chief, Data Processing Section	BFSB, IRP, NINCDS
	Kenneth Elsner	Systems Analyst	BFSB, IRP, NINCDS
	Young Jack Lee	Mathematical Statistician	BFSB, IRP, NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Biometry and Field Studies Branch

SECTION
Data Processing Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of the Medical Studies Database System (MSDS) is to provide a computerized system that facilitates data handling functions with a high degree of automation, that minimizes data collection errors and computer programming, and provides forms-tracking, data updating with automatic audit-trail and user-friendly data retrieval.

Terminal emulation capability has been added to the system to enable intra-computer communications. This capability facilitates the retrieval of a considerable quantity of data from remote computers largely under computer control. Since these procedures require little operator intervention, the number of errors has been significantly reduced and greater quantities of data can be handled.

Additional extensions and enhancements have been made to expand the data-tracking and progress-reporting functions and to expedite the implementation of additional studies on the system. Three clinical studies, the Febrile Seizure study (Z01 NS 02444-06), the Naloxone dose-escalation study and the Pilot Hypervolemic Hemodilution study of Dextran-40 (Z01 NS 02637-02) are currently being managed using this system.

Maintenance and operation of the system will continue and new medical studies may be supported by the MSDS, but the development of the system has been completed and the project is completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02483-05 BFSB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Predictive Value of the EEG in Febrile Seizures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lawrence V. Rubinstein	Mathematical Statistician	BFSB, IRP, NINCDS
Others:	Jonas H. Ellenberg	Deputy Chief	BFSB, IRP, NINCDS
	Karin B. Nelson	Chief, Cerebral Palsy and Other Motor Disorders Section	DNB, CDNDP, NINCDS
	Deborah G. Hirtz	Pediatric Neurologist	DNB, CDNDP, NINCDS
	Martha Griswold	Statistician	BFSB, IRP, NINCDS

COOPERATING UNITS *(if any)*
Cerebral Palsy and Other Motor Disorders Section, DNB, CDNDP, NINCDS;
Pediatric Clinic, University of Skopje, Yugoslavia (Nikola Sofijanov)

LAB/BRANCH
Biometry and Field Studies Branch

SECTION
Mathematical Statistics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.65	0.30	0.35

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This population based study will evaluate the significance of the EEG as a predictor for recurrence of seizures in those children who have had a simple febrile convulsion. Outcome with respect to febrile seizure recurrence and afebrile seizure occurrence will be reported. The evolution of the EEG pattern will be described, and patterns will be correlated with the clinical outcome. The clinical study is being carried out in Skopje, Yugoslavia, at the Pediatric Clinic of the University of Skopje.

The study began in FY'82 and will be completed in FY'88. During FY'85 the data management and quality control systems were revised as needed. By the first quarter of FY'85, approximately 400 patients with a febrile seizure, no prior complex or multiple seizures and with a normal or nonspecific abnormal EEG were registered into the study and began the study protocol and follow-up. An additional 200 patients with a specific abnormal EEG were entered for baseline information and follow-up. Data monitoring, editing and file creation are continuing. Statistical analysis of short-term outcomes and EEG changes will begin in FY'86. Patient accrual was completed in December 1984 and follow-up should be completed on all patients by FY'88.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02411-07 BFSB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Survey of Practice in the Management of Febrile Seizures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Young Jack Lee	Mathematical Statistician	BFSB, IRP, NINCDS
Others:	Jonas H. Ellenberg	Deputy Chief	BFSB, IRP, NINCDS
	Deborah G. Hirtz	Pediatric Neurologist	DNB, CDNDP, NINCDS
	Karin B. Nelson	Chief, Cerebral Palsy and Other Motor Disorders Section	DNB, CDNDP, NINCDS

COOPERATING UNITS *(if any)*
Cerebral Palsy and Other Motor Disorders Section, DNB, CDNDP, NINCDS

LAB/BRANCH
Biometry and Field Studies Branch

SECTION
Mathematical Statistics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.35	0.20	0.15

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A survey of clinical practice in the management of febrile seizures has been conducted using the AMA membership list. The survey questionnaire was sent to a probability sample of 10,000 physicians. The primary data analysis has been completed and a manuscript is in preparation. From the analysis, it was determined how each medical discipline manages children with febrile seizures. Medical factors that are, in themselves, considered sufficient for chronic treatment or consultation were determined for each specialty. Goals of treatment, preferred medicines, whether and why to monitor blood drug levels, and whether and why to hospitalize were analyzed for each specialty. In order to carry out the required analysis, methods for adjusting the effects of concomitant variables for the categorical data analysis were evaluated. The logistic regression was selected as a principal method for the adjustment.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02594-03 BFSB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Factors Predictive of Reading and Writing Skills in the Congenitally Deaf*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Richard F. Raubertas	Mathematical Statistician	BFSB, IRP, NINCDS
Others:	Christy Ludlow	Speech Pathologist	CDP, NINCDS
	Judith Cooper	Speech Pathologist	CDP, NINCDS

COOPERATING UNITS (if any)

Central Institute for the Deaf, St. Louis, MO (Ann Geers);
Gallaudet College, Washington, D.C. (Donald Moores)

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Mathematical Statistics Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project consists of the statistical and data management aspects of this Communicative Disorders Program contract. Tasks include design of data collection and monitoring procedures, and statistical analysis of study data.

The study will examine factors that may be associated with development of reading and writing skills in the congenitally deaf. Study subjects will comprise three groups of deaf 16- to 17-year-olds, with 65 subjects in each group. Each group will include only subjects who received their preschool language training through one of three approaches: aural-oral, total communication, and American Sign Language. Data will be collected on the audiologic, familial, and educational background of the subjects, and on their present language skills. These data will be examined for their association with present reading and writing skills of the subjects.

*[This project is the BFSB/NINCDS support of the CDP contract study NIH-NINCDS-84-19. The project officer is Dr. Christy Ludlow, CDP/NINCDS.]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02489-05 BFSB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Evaluation of Communicative Disorders Information by MEDLINE*†

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Young Jack Lee	Mathematical Statistician	BFSB, IRP, NINCDS
Others:	Christy Ludlow	Speech Pathologist	CDP, NINCDS
	Barbara Reiner	Expert	CDP, NINCDS
	Sylvia Edelstein	Chief, Data Processing Section	BFSB, IRP, NINCDS
	Karlin Richardson	Programmer	BFSB, IRP, NINCDS

COOPERATING UNITS *(if any)*

Communicative Disorders Program, NINCDS

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Mathematical Statistics Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Five information centers participated in an evaluation project in which over 900 participants were enrolled and received MEDLINE services. Information was gathered on the participants' characteristics, information needs and practices prior to participation. After training and receiving MEDLINE services, 80% of the participants completed a post-use evaluation questionnaire. The study demonstrated that among specialists in communicative disorders, those involved in research activities used MEDLINE services most frequently, were most satisfied and saw the greatest need for MEDLINE services. Those involved in clinical services saw less of a need for access to bibliographic services.

The study indicated that most participants used it infrequently, one to two times per year, and therefore forgot how to operate it effectively. Only those primarily involved in research used it frequently enough to report an interest in having direct access to MEDLINE services.

Recommendations were made for the NINCDS staff to encourage the development of self supporting direct access user groups within the scientific community in communicative disorders. This project has been completed.

*[This study is the BFSB/NINCDS portion of a larger contract study entitled: Evaluation of the Effectiveness of Information Services Provided to Specialists in Communicative Disorders by MEDLINE. The project officer is Dr. Christy Ludlow, CDP, NINCDS. Contract numbers are N01-NS-0-2342, N01-NS-0-2343, N01-NS-0-2344, N01-NS-0-2345 and N01-NS-0-2346.]

†Formerly titled "Evaluation of the effectiveness of information services provided to specialists in communicative disorders by MEDLINE."

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02638-02 BFSB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Survey of Major Neurological Disorders in Copiah County, Mississippi

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Dallas W. Anderson	Mathematical Statistician	BFSB, IRP, NINCDS
Others:	Bruce S. Schoenberg	Chief, Neuroepidemiology Branch	IRP, NINCDS

COOPERATING UNITS *(if any)*
University of Mississippi Medical Center, Jackson, MS (Armin F. Haerer)

LAB/BRANCH
Biometry and Field Studies Branch

SECTION
Mathematical Statistics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.75	0.65	0.10

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The primary objective of the project is to establish the prevalence of major neurological and developmental disorders (stroke, epilepsy, cerebral palsy, psychomotor delay, Parkinson's disease, essential tremor, and dementia) in a well-defined population of southern blacks and whites. A secondary objective is to evaluate certain screening questions for possible use in other morbidity surveys.

The background information and methods employed in the study have been published. Prevalence of essential tremor, cerebral palsy, dementia, and Parkinson's disease, noting racial differences, have been published also. Manuscripts on stroke and epilepsy have been submitted for publication. Work is in progress on psychomotor delay and an overview of the disorders of interest.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02652-01 BFSB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Intramural Statistical Collaboration and Consultation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James M. Dambrosia	Chief, Mathematical Statistics Section	BFSB, IRP, NINCDS
Others:	Dallas Anderson	Mathematical Statistician	BFSB, IRP, NINCDS
	Young Jack Lee	Mathematical Statistician	BFSB, IRP, NINCDS
	Richard Raubertas	Mathematical Statistician	BFSB, IRP, NINCDS
	Lawrence Rubinstein	Mathematical Statistician	BFSB, IRP, NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Mathematical Statistics Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.5	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is designed to provide statistical collaboration and consultation for Laboratories and Branches within the Intramural Research Program. Particular consideration is given to statistical planning and design of experiments, statistical analysis of data, and statistical inference. Our collaboration has involved eight laboratiories/branches, and the scope of the studies has ranged from the coordination and statistical management of a complex clinical trial to consultation on the correctness of the statistics used for small laboratory experiments. Examples of studies in the IRP include a randomized clinical trial of W-544, a new drug for the treatment of intractable partial seizures (CMN); an evaluation of efficacy of the drug PGBX for treatment of ischemic stroke in a gerbil model (LNNS and LN); a statistical examination of space-time clustering of Creutzfeldt-Jakob disease in a defined population (LCNSS); an examination of the relation of polio viruses to Alzeheimer's disease (LCNSS); a study of the relationship of viral antibodies to MS (ID); and a study of the dopaminergic modulation of DES-induced proliferation of the anterior pituitary gland of the Fisher 344 rat (ET), and the effect of calcium channel modifiers on prolactin release (ET).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02506-05 BFSB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Antibody Titers in Macacas on Cayo Santiago

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Statistician	(to be assigned when data becomes available)	BFSB, IRP, NINCDS
Others:	William T. London	Chief, Experimental Pathology Section	IDB, IRP, NINCDS

COOPERATING UNITS *(if any)*
Infectious Diseases Branch, IRP, NINCDS; Caribbean Primate Research Center, University of Puerto Rico (Matthew J. Kessler, Project Director)

LAB/BRANCH
Biometry and Field Studies Branch

SECTION
Mathematical Statistics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland, 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project will test for the presence of five viral antibodies in adult and juvenile Macacas on Cayo Santiago, Puerto Rico. There have been no new Macacas introduced into this colony since 1938, at which time testing indicated the presence of antibody in the colony to SV 40, herpes, measles and CMV. The fifth antigen, simian retrovirus D, was added this year. The objective will be to see if at this time this closed colony has lost antibody to the four previously studied antigens and would, therefore, provide an animal population useful for the testing of related strains of viruses. If this colony is shown to be negative for simian retrovirus, it would be very useful in the study of simian AIDS. To date four of the five troups of monkeys on Cayo Santiago have been trapped and bled. The remaining troup will be trapped, bled and all serological analysis completed by late 1986, at which point statistical analysis of the data will begin.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02486-05 BFSB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Statistical Models of In Vitro Mutagenicity Assays

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Young Jack Lee	Mathematical Statistician	BFSB, IRP, NINCDS
Others:	William J. Caspary	Biochemist	NTP, NIEHS

COOPERATING UNITS (if any)
National Toxicology Program, National Institute of Environmental Health Sciences

LAB/BRANCH
Biometry and Field Studies Branch

SECTION
Mathematical Statistics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Chemically-induced genetic damages of cells (mammalian or submammalian) in vitro are observable by allowing the cells to express their DNA damage and the progenies with locus-specific mutation to be selected and form colonies.

A report describing the statistical analysis method based on a biological model of the lymphoma cell assay (Mutation Research 113: 417-430, 1983) is under preparation. A nonparametric statistical method to analyze the Ames test data has been developed and its efficacy has been compared to that of a parametric method. A paper describing the nonparametric method is under preparation. All scientific reports should be submitted for publication in FY'85.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02592-03 BFSB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Central Nervous System Metastases from Lung Cancer*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lawrence V. Rubinstein	Mathematical Statistician	BFSB, IRP, NINCDS
Others:	Mitchell H. Gail	Medical Statistician	BB, DCE, NCI
	Steven Piantadosi	Medical Staff Fellow	BB, DCPC, NCI

COOPERATING UNITS *(if any)*

Biometry Branch, DCPC, NCI; Illinois Cancer Council; Mayo Clinic; Seattle Cancer Group; Toronto Cancer Group; UCLA Medical Center

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Mathematical Statistics Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	0.00

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Lung Cancer Study Group (LCSG) has determined that central nervous system, in particular brain, metastases account for approximately 25% of the first recurrences in Stage I lung cancer. BFSB has collaborated on the analysis of the LCSG data to determine the relationships of recurrence in the CNS to cell type, tumor classification, nodal involvement, and other prognostic factors. The findings have been published and the project is completed.

*[In order to accomplish this study BFSB is using the data generated by NCI contract Z01-CP-04260-23B entitled: "Consultation on Clinical Trials."]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02591-03 BFSB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Reye's Syndrome Study

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Young Jack Lee	Mathematical Statistician	BFSB, IRP, NINCDS
Others:	Anita Chu	Expert	IDB, IRP, NINCDS

COOPERATING UNITS *(if any)*
Infectious Diseases Branch, IRP, NINCDS

LAB/BRANCH
Biometry and Field Studies Branch

SECTION
Mathematical Statistics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.10	0.05	0.05

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Infectious Diseases Branch studied salicylate metabolism, other clinical chemistries and histocompatibility antigens in families with Reye's Syndrome patients who have completely recovered from the syndrome. BFSB was responsible for all statistical components of the study including design, data analysis and statistical modeling of the clinical chemistry data.

Five survivors and their unaffected family members were studied. This study showed significantly higher antibody levels to Influenza A and varicella, further supporting the importance of these viral infections in the etiology of the syndrome. It did not show an association between RS and 1) abnormal salicylate metabolism, 2) abnormal helper to suppressor T cell ratios and lymphocyte stimulation responses, 3) specific HLA type, and 4) permanent neuropsychologic sequelae.

A paper has been prepared for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02114-12 BFSB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Etiology and Natural History of Convulsive Disorders and Cerebral Palsy*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jonas H. Ellenberg	Deputy Chief	BFSB, IRP, NINCDS
Others:	Karin B. Nelson	Chief, Cerebral Palsy and Other Motor Disorders Section	DNB, CDNDP, NINCDS
	Deborah Hirtz	Pediatric Neurologist	DNB, CDNDP, NINCDS

COOPERATING UNITS *(if any)*
Cerebral Palsy and Other Motor Disorders Section, DNB, NDP, NINCDS

LAB/BRANCH
Biometry and Field Studies Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.5	0.3

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study examines the relationship between perinatal and early postnatal factors and the occurrence of seizure disorders and cerebral palsy in childhood. The project derives from the data of the Collaborative Perinatal Project, a large prospectively-followed population (approximately 60,000 mothers, with their children followed to seven years of age). The univariate screen of maternal, obstetric and pediatric risk factors, demographic analyses and studies of natural history have been completed. The major focus this fiscal year has been on the multivariate assessment of the data bank which has been substantially completed, including correlation and regression analyses relating to the etiology of both disorders. Final manuscripts in each area are in progress, including pre and postnatal predictors of both disorders.

*[This study is the BFSB/NINCDS portion of larger studies entitled: Convulsive Disorders Data Analysis Group, and Cerebral Palsy Data Analysis Group. The Principal Investigator for these studies is Dr. Karin B. Nelson, Chief, Cerebral Palsy and Other Motor Disorders Section, DNB, CDNDP, NINCDS.]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02312-09 BFSB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Maternal Infection Study*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jonas H. Ellenberg	Deputy Chief	BFSB, IRP, NINCDS
Other:	John L. Sever	Chief	IDB, IRP, NINCDS
	Martha Griswold	Statistician	BFSB, IRP, NINCDS
	Anita Ley	Microbiologist	IDB, IRP, NINCDS
	Dorothy Edmonds	Clinical Nurse	IDB, IRP, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	0.10	0.05

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Analysis of the Collaborative Perinatal Project (CPP) data continues in the area of maternal infection. (The CPP is a prospective study of approximately 60,000 gravidae and the follow-up of their children through the seventh year of life.) The relationship of maternal infection during pregnancy with the later status of the child is being examined using both clinical and serologically-confirmed infections in the mother.

Two primary methodologies have been used, a prospective and a case control approach. A prospective assessment of risk of specified childhood outcome was used for all clinically confirmed infections. Since serological confirmation of all of the common infections occurring during pregnancy on all women in the project would not have been feasible, a case control design has been implemented assessing the titer of 11 antigens in women with abnormal children, in comparison with matched control women with normal children. Special studies of specific infections such as condylomata and toxoplasmosis are in progress or have been completed and a descriptive study of the distribution of titers and frequency of seroconversions by race and age of gravidae for various antigens in a population of pregnant women has been completed. The prospective serological study of toxoplasmosis and its relationship with pregnancy outcome, based on the first 23,000 pregnancies in the study, has shown increases in the risk of deafness, microcephaly and low IQ among children born to women with high maternal antibody to toxoplasmosis.

*[This study is the BFSB/NINCDS portion of a larger study entitled: Perinatal Infections Causing Damage to the Child - Collaborative Perinatal Project, Z01 NS 00402-29 ID. The principal investigator on the overall study is Dr. John L. Sever, Chief, IDB, IRP, NINCDS.]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02505-05 BFSB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Headache in Pregnant Women

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ta-Chuan Chen	Mathematical Statistician	BFSB, IRP, NINCDS
Other:	Karin Nelson	Chief, Cerebral Palsy and Other Motor Disorders Section	DNB, CDNDP, NINCDS
	Sylvia Edelstein	Chief, Data Processing Section	BFSB, IRP, NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.6	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project investigates the relationship between migraine headache and other diseases based on the data collected from the large group of gravidae in the Collaborative Perinatal Project. Subgroups of women characterized by the absence and presence of migraine and other recurrent headaches prior to or during pregnancy, have been identified. Characteristics of these subgroups are being investigated on a variety of demographic, sociological, medical and obstetric factors, and the association of headache with other disorders is being examined. Preliminary results have shown that pregnant women with a migraine history had higher rates of other symptoms and illnesses than women without a migraine history. Children of mothers with a history of migraine appear to have higher incidence of seizures and some infectious and allergic diseases than children born to mothers in the nonmigraine group. More intensive statistical analyses are being carried out to examine the apparent associations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02497-05 BFSB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Indo-U.S. Study of Head Injury

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Selma C. Kunitz	Chief, Computer Applications Section	BFSB, IRP, NINCDS
Other:	Cynthia R. Gross	Biostatistician	BFSB, IRP, NINCDS
	Christine L. Wolf	Programmer	BFSB, IRP, NINCDS

COOPERATING UNITS *(if any)*

University of VA Dept. of Neurosurgery, Charlottesville, VA
All-India Institute of Medical Science, New Delhi, India

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Computer Applications Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	0.10	0.05

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Information on head-injured persons has been collected in independent research efforts in Charlottesville, Virginia, and in New Delhi, India. A preliminary review of these data collection efforts has indicated significant overlap in the type of information collected. Analysis will identify differences and similarities between these head-injured populations, and determine the feasibility of prospective cooperative association for the study of head injuries.

The Government of India has approved the research proposal and has allocated 767,000 rupees for the three-year Indian portion of the collaborative study.

The India data have been entered into the NIH Computer System and relevant Charlottesville data have been transferred to NIH. Data analyses have begun and a report on the pilot phase is being prepared.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02639-02 BFSB

PERIOD COVERED

September 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antecedents and Consequences of Premature Rupture of Membranes in Pregnancy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Richard F. Raubertas Mathematical Statistician BFSB, IRP, NINCDS

COOPERATING UNITS (if any)

Obstetrics and Gynecology, George Washington University Medical Center (John Grossman and Goldee Gross)

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Mathematical Statistics Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project consists of the statistical aspects of a study initiated at the George Washington University Medical Center. Primary tasks include computerization and statistical analysis of study data.

Data have been collected on the mothers and infants involved in about 135 cases of premature rupture of membranes (PROM) seen at the GWU Medical Center. Information available includes demographic variables, some aspects of the mother's medical history, various aspects of the labor and delivery, and the immediate post-delivery course of the mother and infant. Those areas of particular interest are the demographic composition of the PROM patients, the relationship between PROM and maternal infection during pregnancy, and the relationship between length of interval from PROM to delivery and various post-delivery complications. These complications include intraventricular hemorrhage and respiratory distress syndrome in the infant, and infections in both mother and infant.

Information from this study will be used to plan possible clinical trials of medical interventions in PROM.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02653-01 BFSB

PERIOD COVERED

June 1, 1985 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Course and Prognosis Associated with Visual and Hearing Impairment

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Frances M. Baker	Psychiatrist/Epidemiologist	BFSB, IRP, NINCDS

COOPERATING UNITS (if any)

Center for Epidemiologic Studies, Division of Biometry and Epidemiology, NIMH (Eve Mościcki); Chief, Epidemiology, Demography and Biometry Program, NIA (Lon White).

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Mathematical Statistics Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	0.15	0.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Utilizing the NHANES I data (1971-1975), persons with visual and hearing impairment will be divided into several levels of impairment. The NHANES Follow-up Study (1982-1984) will be used to determine the specific outcomes. Specific variables to be considered include the number of hospitalizations between 1971 and 1984, the number and types of associated diagnoses both medical and psychiatric, and the individual's functional level. The focus of this investigation is to describe the course and prognosis associated with visual and hearing impairment and to identify populations at increased risk for deterioration in their functional level. Descriptive and analytic approaches are to be used in interpreting the data.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 NS 02651-01 BFSB

PERIOD COVERED

June 1, 1985 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Senile Dementia Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Frances M. Baker Psychiatrist/Epidemiologist BFSB, IRP, NINCDS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Mathematical Statistics Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	0.15	0.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In conjunction with the Baltimore site of the Epidemiologic Catchment Area Survey of NIMH, NINCDS funded a detailed dementia workup on those persons in the elderly sample identified with dementing illness, by comprehensive psychiatric examination. The detailed dementia workup included a neurologic examination and comprehensive laboratory studies which included thyroid function tests, electrolytes, BUN and glucose, B12 and folate levels, calcium and phosphorous levels, syphilis serology, urinalysis, chest X-ray, EKG, EEG, and CT scan. The foci of this investigation are (1) to explore the limitations of the Mini-Mental-State-Examination (MMSE) as a screening instrument, (2) to explore which components of the comprehensive dementia workup provide the highest specificity when used as a dementia screening test, (3) to approach a screening model for dementia with a sensitivity and specificity improved beyond that of the MMSE, and (4) to examine the usefulness of this model in other elderly populations.

For the confirmed cases of dementia, additional information was gathered on the social and economic impact upon the caregivers. We are considering the feasibility of an investigation which will assess this impact upon the caregivers.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02596-03 BFSB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Data Bank Maintenance Center

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Christine Wolf	Programmer Analyst	BFSB, IRP, NINCDS
Others:	Ella Maneely	Programmer	BFSB, IRP, NINCDS
	Selma C. Kunitz	Chief, Computer Applications Section	BFSB, IRP, NINCDS
	Josh Barwick	Programmer	BFSB, IRP, NINCDS

COOPERATING UNITS *(if any)*

RLR & Associates, Inc., Fairfax, Virginia

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Computer Applications Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.0	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Until October, 1984 the computer and data management aspects of the Stroke Data Bank and Traumatic Coma Data Bank included three entities: 1) a Data Bank Maintenance Center (DBMC) supported by a contract with Beth Israel which provided storage and retrieval support for the combined data bases in each of the data banks 2) a "front-end" micro-computer system supported by a contract with RLR Associates and 3) CAS which coordinated all of the data management aspects of the projects. The contract for a DBMC at Beth Israel was terminated on September 30, 1984, at the convenience of the government, and there has been a restructuring of the described activities.

The coordination of the data management activities for the data banks, as well as design of the data storage, data exploration, and system enhancements are the responsibility of CAS, which is acting now as the DBMC or coordinating center for the data banks. The data now physically reside at NIH (DCRT) in SAS data sets, after they are transmitted from the clinical centers. The RLR Associates' contract workscope has been expanded to include actual programming of the software for DCRT and for transmission of data from the centers to DCRT. RLR Associates will maintain the front-end aspects of the system. In addition, the analytic retrieval functions formerly supported by the Beth Israel DBMC are being transferred to the individual clinical center sites.

A patient tracking system was designed, developed and implemented for the Stroke Data Bank. This system, in which data are directly entered by the clinical centers into DCRT, monitors the flow of patients from entry into the study, through follow-up. A similar system is in the design phase for the Traumatic Coma Data Bank.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02443-06 BFSB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Offline Data Entry System for Stroke and Coma Projects

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Christine Wolf	Programmer Analyst	BFSB, IRP, NINCDS
Others:	Ella Maneely	Programmer	BFSB, IRP, NINCDS

COOPERATING UNITS (if any)

RLR & Associates, Inc., Fairfax, VA

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Computer Applications Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This work has been subsumed under Project Z01-NS-02596-03.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02516-04 BFSB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Traumatic Coma: Epidemiological Characteristics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Cynthia R. Gross	Biostatistician	BFSB, IRP, NINCDS
Others:	Selma C. Kunitz	Chief, Computer Applications Section	BFSB, IRP, NINCDS
	Christine Wolf	Programmer	BFSB, IRP, NINCDS

COOPERATING UNITS *(if any)*

Consultant (Rene K. Kozloff)

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Computer Applications Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.20	0.05

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The pilot Traumatic Coma Data Bank (N01-NS-9-2306,7,8,9) collected information on 581 patients with severe head injuries, drawn from six centers in the United States. These data are being analyzed to identify patterns of injury and type of accident as they vary from center to center, by patient demographic characteristics, season and time of day. By profiling the characteristics of the 58 children in the data bank, it was found that pedestrian accidents (i.e., children who were struck by motor vehicles) were the most frequent cause of injury and that falls were most common among infants and toddlers. The case frequency sex ratio varied with age, being 2:1 (male excess) in children, almost 4:1 in the middle ages, and about 1:1 in the 60-and-older age group. Case fatality rates differed by age, but not by sex.

A study submitted for publication focused on the age groups 15-24 years. The typical head injury victim was a young man between the ages of 15 and 24. Sex differences between injury victims in this age group include differences in mechanism of injury, role (driver, occupant, pedestrian) of the injured person, and in alcohol use at the time of accident.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 02408-07 BFSB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Epidemiologic Research with Clinical Data Banks*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Cynthia R. Gross	Biostatistician	BFSB, IRP, NINCDS
Others:	Selma C. Kunitz	Chief, CAS	BFSB, IRP, NINCDS
	Irene G. Fishman	Statistician	BFSB, IRP, NINCDS
	Christine L. Wolf	Programmer	BFSB, IRP, NINCDS
	Margaret Meadows	Statistical Assistant	BFSB, IRP, NINCDS
	David Shinar	Psychologist	BFSB, IRP, NINCDS

COOPERATING UNITS (if any)

Depts. of Neurology: B.U. School of Medicine, Michael Reese Hospital, New York Neurological Institute and U.Md. Depts. of Neurosurgery: U.Va, M.C.V., U. Texas at Galveston and U.C.S.D.

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Computer Applications Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Work on determining which epidemiologic approaches are most appropriate for use with clinical data banks was begun in conjunction with the Pilot Stroke and Traumatic Coma Data Bank Networks and is continuing with the full phases of these projects (N01-NS-2-2302, 2398, 2399, N01-NS-5-2384; N01-NS-3-2339-2342). This project has focused upon quality assurance methods and epidemiological considerations in the data collection, analysis and interpretation of data bank results.

Work completed in FY '85 included quality assurance studies on the validity of a depression symptoms scale which was developed for epidemiologic surveys (CES-D), for use with the Stroke Data Bank in the assessment of the incidence and severity of depression in Stroke Data Bank patients, and a review of coding accuracy in use of the Abbreviated Injury Scale (A.I.S.) to record multiple trauma to the traumatic coma patients. Data collection for a validity study of the telephone assessment of Activities of Daily Living for stroke patients is scheduled to begin in June, 1985. Analysis is planned for Summer and Fall, 1985.

*[Formerly "Clinical Data Banks as a Resource for Epidemiologic Research"]

PHS 6040 (Rev. 1/84)

READEX FICHE

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02595-03 BFSB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Methodological Aspects of Data Banks

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Irene G. Fishman	Statistician	BFSB, IRP, NINCDS
Other:	Selma C. Kunitz	Chief, Computer Applications Section	BFSB, IRP, NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Computer Applications Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Data Banks have been developed in Stroke and Traumatic Coma. Organizing the principles for establishing a data bank which describes a neurologic condition requires proposing and testing new concepts of data management. This project has analyzed the underlying organizational and methodological principles which are necessary for optimal functioning of a data bank, has developed a data bank model, and will disseminate information regarding this model.

The methodology employed by the data banks includes innovative techniques, such as interactive, on-site data entry, and local edit checking of data. Since the data banks consist of multiple clinical centers, which collaborate and pool data, stringent techniques are required to ensure consistent data collection. A data base management system is necessary to handle the hundreds of variables involved. Information on this methodology is being disseminated by presentations at seminars, papers, meetings and conferences. A Stroke Data Bank Workshop will be sponsored at the Stroke Council of the American Heart Association in 1986. In addition, consultation with other Institutes and with academic medical centers on data bank methodology for chronic disease continues. For example, the Arthritis Institute has implemented a data bank on liver transplants based on the organizational and data management principles of the Stroke and Coma Data Banks.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02498-05 BFSB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Observer Agreement Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Cynthia Gross	Biostatistician	BFSB, IRP, NINCDS
Others:	Selma C. Kunitz	Chief, CAS	BFSB, IRP, NINCDS
	Irene G. Fishman	Statistician	BFSB, IRP, NINCDS
	Karlin I. Richardson	Programmer	BFSB, IRP, NINCDS
	Christine L. Wolf	Programmer	BFSB, IRP, NINCDS
	Margaret A. Meadows	Statistical Assistant	BFSB, IRP, NINCDS
	David Shinar	Psychologist	BFSB, IRP, NINCDS

COOPERATING UNITS *(if any)*

Depts. of Neurology: B.U. School of Medicine, Michael Reese Hospital, N.Y. Neurological Institute, U.MD School of Medicine. Depts. of Neurosurgery: U.Va., M.C.V., U.T. at Galveston and U.C.S.D.

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Computer Applications Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To demonstrate that data from the Stroke and Traumatic Coma Data Banks are reliable, studies of inter-observer agreement have been implemented. These studies include a pilot study of agreement among CT Scan readers in the pilot Traumatic Coma Data Bank (N01-NS-3-2306,2307,2308,2309, BFSB), and studies of variations in neurological examination, diagnosis and CT scan reading for the Stroke Data Bank (N01-NS-2-2302,2398,2399, N01-NS-5-2384). Four studies have been initiated to date.

A study of CT measurements is being planned for the Coma Data Bank. A manuscript on observer agreement in stroke diagnosis has been written and is being submitted for publication. A manuscript on observer agreement in CT readings of stroke anatomy is in preparation.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02598-03 BFSB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Complications, Recurrence, and Outcome: Stroke Data Bank

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Cynthia R. Gross	Biostatistician	BFSB, IRP, NINCDS
Others:	Irene G. Fishman	Statistician	BFSB, IRP, NINCDS

COOPERATING UNITS *(if any)*

Department of Neurology, Boston U. Medical Center, Boston, Massachusetts

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Computer Applications Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The majority of stroke patients will survive the acute episode; some will recover to their pre-stroke levels of functioning; and others will be disabled to some degree. Complications following stroke may result from the insult itself or be related to diagnostic or therapeutic methods used in stroke management. Complications may prolong a patient's hospital stay and affect his ultimate outcome. Data from the Stroke Data Bank (N01-NS-2-2302, N01-NS-2398, 2399, N01-NS-5-2384), a prospective, multicentered, study of hospitalized stroke patients, will be used to profile the complications-prone patient. Socio-demographic and clinical data, including age, sex, location and type of stroke, severity and type of initial deficit(s) will be compared with occurrence of complications such as seizures, visceral bleeding and stroke recurrence to characterize those patients who experience complications, as well as to contrast their course with a similar group of stroke patients who differ in that they do not have complications. The clinical course of those patients with complications is being studied in order to determine the impact of complications on outcome. Data collection began in June 1983, and as of May 1985, over 1,300 cases were enrolled. Data analysis will take place in FY '86.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02599-03 BFSB

PERIOD COVERED

October 1, 1984 through September 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Behavioral Factors Influencing Recovery from Stroke

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Selma C. Kunitz — Chief, Computer Applications Section — BFSB, IRP, NINCDS

COOPERATING UNITS *(if any)*

Boston University (Philip Wolf); University of Maryland (Tom Price); Michael Reese Medical Center (Lou Caplan); University of South Alabama (Jay Mohr)

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Computer Applications Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.20	0.20	0.00

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to better comprehend the factors influencing recovery from Stroke, behavioral factors were studied, utilizing the first 436 patients of the Stroke Data Bank population (N01-NS-2-2302, N01-NS-2-2398, 2399, N01-NS-5-2384). Specifically, two dimensions of social support were examined with respect to stroke outcome. The two dimensions are source (family and institutional) and type (affective and instrumental). Patients are stratified by stroke severity. Definition of outcome includes Activities of Daily Living (ADL) and social functioning. Data collection for this project began in July 1983. Outcome differences at three months were associated with family support and friend support, depending upon stroke severity. Analysis will continue on a file of over 800 patients currently in the central computer. Outcome at six months and one year will also be examined.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02590-03 BFSB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Statistical Studies on the Stroke Data Bank*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	James M. Dambrosia	Chief, Mathematical Statistics Section	BFSB, IRP, NINCDS
Others:	Lawrence V. Rubinstein	Mathematical Statistician	BFSB, IRP, NINCDS

COOPERATING UNITS (if any)
University of Maryland; Boston University; Michael Reese Hospital; Columbia University

LAB/BRANCH
Biometry and Field Studies Branch

SECTION
Mathematical Statistics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.55	0.50	0.05

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project currently includes four studies each of which is a component of the Stroke Data Bank or its precursor, the Pilot Stroke Data Bank. The studies are: (1) Evolving Stroke. Using demographic, history, clinical and laboratory data, this study describes the temporal course of stroke-in-evolution and attempts to identify factors that cause or contribute to evolution. (2) Stroke Diagnosis. A set of diagnostic algorithms for stroke classification based on laboratory and clinical findings were developed during the pilot project. The usefulness of the algorithms is being evaluated for differentiating etiology and predicting outcome. Plans for analyses have been formulated. (3) Utility of diagnostic tests. A variety of diagnostic tests (including angiography, CT scanning and noninvasive cardiac and vascular tests) are available for the study of the stroke patient. We intend to investigate the utility of each of these tests in establishing stroke cause and examine the utility of these tests in predicting survival rate, degree of recovery, and risk of stroke recurrence. Study designs and analyses plans have been formulated for this study. (4) Prognostic factors for 30-day mortality. This study determines a set of prognostic factors available shortly after hospitalization for ischemic strokes that are predictive of 30-day mortality. A logistic regression model has been developed based on 620 stroke cases with 52 deaths within 30 days post onset available from the pilot project. Factors (112 in all) were initially screened by univariate statistical methods and those screened positive were used multivariately in a logistic model. Cross validation of the model will be accomplished on the current Stroke Data Bank.

*This project includes projects previously reported as Z01 NS 02587-03 BFSB and Z01 NS 02492-05 BFSB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02492-05 BFSB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Stroke Diagnosis and Prognosis Based on the NINCDS Data Bank

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lawrence V. Rubinstein	Mathematical Statistician	BFSB, IRP, NINCDS

COOPERATING UNITS *(if any)*

Boston University Medical Center; New York Neurological Institute; University of Maryland Hospital; Michael Reese Hospital; Beth Israel Hospital

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Mathematical Statistics Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is now being reported as a part of the project "Statistical Studies on the Stroke Data Bank", as Project Z01 NS 02590-03 BFSB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02587-03 BFSB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Utility of Diagnostic Tests in Predicting Stroke Mechanism and Outcome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Dallas W. Anderson	Mathematical Statistician	BFSB, IRP, NINCDS

COOPERATING UNITS *(if any)*
Michael Reese Hospital and Medical Center, Chicago, IL (Louis R. Caplan)

LAB/BRANCH
Biometry and Field Studies Branch

SECTION
Mathematical Statistics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is now being reported as a part of the project "Statistical Studies on the Stroke Data Bank", as Project Z01 NS 02590-03 BFSB.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02500-05 BFSB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Polymyositis/Dermatomyositis Study

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Irene G. Fishman Statistician BFSB, IRP, NINCDS

COOPERATING UNITS *(if any)*
Neurological Center of the Pennsylvania Hospital (Christopher Clark)

LAB/BRANCH
Biometry and Field Studies Branch

SECTION
Computer Applications Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	0.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The low incidence of myositis and its chronic course necessitates collaboration of a number of investigators. This project involved consultation by BFSB staff to a group of neurologists who were planning the collection of clinical information on myositis patients. An initial set of data items for collection was proposed, and forms were designed to enter data on demographic information, initial evaluation, and subsequent follow-up. These forms were distributed to interested researchers, and refinements were made incorporating experience with their use. BFSB staff acted in a consultative role to this extramural group of investigators. This project has been completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02404-07 BFSB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

National Survey of Chronic and Debilitating Headache

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Frederic D. Weinfeld	Chief, Surveys and Demographic Studies Section	BFSB, IRP, NINCDS
Others:	Ta-Chuan Chen	Mathematical Statistician	BFSB, IRP, NINCDS
	Dallas W. Anderson	Mathematical Statistician	BFSB. IRP, NINCDS

COOPERATING UNITS *(if any)*

National Center for Health Statistics; California Medical Clinic for Headache; Cleveland Clinic; Diamond Headache Clinic; Headache Research Foundation

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Surveys and Demographic Studies Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.10	0.05	0.05

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purposes of this study are to collect data on severe headache in order to measure the prevalence and to describe the demographic characteristics of the major types of headache. To this end a survey of the general population has been designed. A survey questionnaire, which includes sections on demography, descriptive headache features, medical information, and history, has been developed. The data will also be used to identify and assess the etiological and environmental factors associated with the major idiopathic headache types.

The study was designed in two parts: a feasibility study and an area survey. The feasibility study has been completed. Telephone interviews have been conducted with the patients from four headache clinics. The questionnaire data have been processed together with information abstracted from the physician records about the headaches. The planning and design of the area survey has been completed. The area survey will not be funded and this study is thereby completed.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02636-02 BFSB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Classification of Headache Types Based on Symptomatology and Features

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Robert Richter Mathematician BFSB, IRP, NINCDS

Other: Frederic D. Weinfeld, Chief, Surveys and Demographic Studies Section, BFSB, IRP, NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Surveys and Demographic Studies Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.10	1.00	0.10

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Three studies of headache features in migraine, cluster and tension headaches were developed based on the data collected from a feasibility study for a survey of headache (Z01 NS 02404-07). The feasibility study involved 243 patients from four headache clinics. The first study used four group discriminant analysis to develop statistically a parsimonious set of headache features and symptoms which could be used to correctly classify a high percentage of patient headaches into one of the four headache types of common migraine, classical migraine, cluster or tension headache. A second study using factor analysis, on the combined group of headaches, attempted to isolate patterns of symptoms and features of headaches which mirror clinical descriptions of the four headache types above. The study yielded indeterminate results. The third study, describes for each headache type, the frequency of and interrelationships among headache symptoms and features, and relates precipitating factors such as eyestrain, menstruation, etc., to these patterns.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02494-05 BFSB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Prevalence of Multiple Sclerosis in Colorado

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Herbert M. Baum	Demographer	BFSB, IRP, NINCDS
Others:	Sandra Calingo	Computer Aide	BFSB, IRP, NINCDS

COOPERATING UNITS *(if any)*

The Rocky Mountain Multiple Sclerosis Center, University of Colorado School of Medicine

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Surveys and Demographic Studies Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.15	0.10	0.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Rocky Mountain Multiple Sclerosis Center is one of a few centers devoted solely to the care of patients with multiple sclerosis, and is the only center of its type in the State of Colorado. Using records from the Center, the local chapter of the National Multiple Sclerosis Society, hospital records, and physician records we estimated the prevalence of multiple sclerosis for Weld and Larimer Counties, after accounting for duplicate cases.

Crude point prevalence for the two-county region was 84 per 100,000. Methodological results revealed that the highest yield sources of cases were the MS service organizations and the neurology practice chart reviews. Prevalence surveys which neglect these sources may underestimate MS prevalence by as much as 20 to 40%.

A manuscript "Higher than expected prevalence of multiple sclerosis in Northern Colorado: Dependence on methodologic issues," has been submitted for publication.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02586-03 BFSB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

An Examination of Multiple Cause of Death Data for Stroke

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Herbert M. Baum Demographer BFSB, IRP, NINCDS

COOPERATING UNITS (if any)

Center for Population Studies, Duke University

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Surveys and Demographic Studies Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has three main goals. First to determine whether a change in the coding of a stroke on death certificates as underlying versus as an associated cause of death is partially responsible for the large decline in the rates of stroke mortality as calculated from the underlying cause of death. Next, to construct life tables and approximate the impact of eliminating stroke as a cause of death; and lastly, to examine the pattern of multiple causes of death which occur from stroke.

Computer tapes, issued by the National Center for Health Statistics, containing all death certificates in the United States for the period 1968-1978 were used. All certificates where stroke (ICDA-8 Codes 430-438) was listed as either an underlying or associated cause of death were selected for study. The data were then tabulated by age, race, and sex. Life tables were constructed to estimate the change in life expectancy if stroke were eliminated as a cause of death. An examination of disease pairs (underlying and associated) was also undertaken.

During FY '84 an article on "CVD Mortality, 1968-1978; Observations and implications" was published. Another article investigating the diseases which appear in conjunction with stroke on the death certificate entitled "Cerebrovascular disease mortality: The relationship of underlying and associated causes of death," has been submitted for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02504-05 BFSB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Epidemiological Study of Pain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ta-Chuan Chen Mathematical Statistician BFSB, IRP, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to evaluate the overall and age-specific incidence rates of various chronic pain syndromes by developing a statistical technique to estimate incidence rates from age of onset data. The incidence rates of disabling and/or severe headache were evaluated with data obtained from a Midwest non-clinical population survey. The use of incidence and prevalence rates in the estimation of length of illness due to headache has been examined. A report of the results of this study has been prepared and will be submitted for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02515-04 BFSB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of Hearing Disorders Among the Aged

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Eve K. Mościcki	Scientist	BFSB, IRP, NINCDS
Others:	Herbert M. Baum	Demographer	BFSB, IRP, NINCDS

COOPERATING UNITS *(if any)*

Communicative Disorders Program; National Heart Lung and Blood Institute

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Surveys and Demographic Studies Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.00	.00	.00

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are to describe the prevalence of hearing loss in the Framingham cohort by demographic characteristics, to investigate the relationship between the severity of hearing loss and otologic risk factors, and to examine possible relationships between hearing loss and cardiovascular risk factors and events. Hearing data collected during Cycle 15 of the Framingham Heart Study (1978-1979) have been analyzed to estimate the prevalence of hearing loss among the Framingham cohort. The risk factors that might be associated with hearing loss found in this population have been examined. The main demographic and otologic factors associated with hearing loss were advanced age, male sex and history of hearing loss associated with illness.*

*This project was completed in FY '84. A manuscript, Hearing loss in the elderly: An epidemiologic study of the Framingham Heart Study cohort. Ear Hear. 6(4): 184-190, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02490-05 BFSB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Research in Statistics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James M. Dambrosia	Chief, Mathematical Statistics Section	BFSB, IRP, NINCDS
Others:	Young Jack Lee	Mathematical Statistician	BFSB, IRP, NINCDS
	Dallas W. Anderson	Mathematical Statistician	BFSB, IRP, NINCDS
	Jouas H. Ellenberg	Deputy Chief	BFSB, IRP, NINCDS
	Lawrence V. Rubinstein	Mathematical Statistician	BFSB, IRP, NINCDS
	Richard F. Raubertas	Mathematical Statistician	BFSB, IRP, NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Biometry and Field Studies Branch

SECTION
Mathematical Statistics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.75	0.75	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project addresses statistical problems generated from collaboration with scientists in other program areas and general statistical problems of current interest. This project is a continuing activity of the Section on Mathematical Statistics. Papers have been published in FY'85 on the following statistical subjects: tests of monotone trend for k Poisson means, a diffusion approximation of absorption probabilities for binomial random walks in the presence of linearly varying boundaries, modeling covariate dependence of binary sequences, pooled adjacent violators, and general tests of trend for count data. Other work in progress includes the design and analysis of Phase II clinical trials, nonparametric tests with quadratic alternatives, statistics for the evaluation of space-time clustering of disease, modeling of residual treatment effect for k-period two-treatment crossover designs, statistical inference under inequality constraints, adjustments for covariates in the analysis of categorical data, the influence of missing data on statistical models determined by variable selection procedures, the effects of misclassification of exposure variables on case-control studies, the use of area surveys in epidemiological research, and sampling strategies for rare diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02517-04 BFSB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Statistical Methodology for the Measurement of Pain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ta-Chuan Chen	Mathematical Statistician	BFSB, IRP, NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project investigates the statistical problems involved in the measurement of experimental and clinical pain. (1) A study has been conducted to investigate the statistical technique used in deriving psychophysical measurements of pain. A report has been prepared for this work dealing with the interrelationship of sensory-decision-theory measures such as d' and β and nonparametrical measurement indices, such as p(A), Hodo's percent bias and MacNicol's index of response bias, β. The investigation of this part of the work is completed. The report of this study was presented at the 4th World Congress on Pain in 1984. A manuscript is in preparation. (2) A study of statistical quantification of the temporal characteristics of persistent, episodic pain such as migraine headache is currently being developed. A group of measurements for this type of pain has been selected for investigation.

TAB 14 -- DEVELOPMENTAL AND METABOLIC NEUROLOGY BRANCH -- (DMNB)

ANNUAL REPORT

October 1, 1984 through September 30, 1985
Developmental and Metabolic Neurology Branch
National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT

October 1, 1984 through September 30, 1985
Developmental and Metabolic Neurology Branch, IRP
National Institute of Neurological and Communicative Disorders and Stroke

Roscoe O. Brady, Chief

Principal activities of the Branch concern the following areas of investigation: 1. Examination of pathways of sphingolipid and mucopolysaccharide synthesis and catabolism and elucidation of enzymatic abnormalities in human metabolic disorders. 2. Clinical studies of neurogenetic diseases.
3. Production of cellular and animal models of disorders of metabolism.
4. Studies of the structure and cell biology of lysosomal enzymes.
5. Elucidation of the molecular basis of lysosomal storage disorders.
6. Development of therapy for patients with heritable diseases.
7. Transmembrane signalling and the role of glycoconjugates in this process.
8. The role of glycolipids and glycoproteins in the development of the nervous system, autoimmune phenomena, and in demyelinating diseases.

I. HEREDITARY METABOLIC DISORDERS

A. Demonstration of a Metabolic Defect in Type C Niemann-Pick Disease.

Nearly two decades have passed since the discovery of the deficiency of sphingomyelinase in tissues from patients with Niemann-Pick disease Type A (infantile onset of hepatosplenomegaly along with extensive CNS damage) and Type B (organomegaly without brain involvement). A number of patients have been classified as Type C Niemann-Pick disease who have somewhat later onset of organomegaly and CNS deterioration. There is only a moderate accumulation of sphingomyelin in the tissues from these patients accompanied by an elevation of cholesterol. Sphingomyelinase activity in cultured skin fibroblasts derived from these individuals is usually in the heterozygous range but may even be normal in some specimens. The activity of this enzyme is not decreased in parenchymal tissues. Based on the discovery of impaired esterification of exogenous cholesterol in tissues and fibroblasts obtained from the mutant BALB/c mouse colony we established, this reaction was examined in cultured fibroblasts from patients with Type C Niemann-Pick disease. There was a dramatic decrease in esterification of cholesterol in cultured skin fibroblasts derived from most of these patients. It is believed that this lesion is the primary metabolic defect in many of the patients in this category. However, it is equally apparent that this is not a clinically homogeneous group and the correct clinical category and metabolic derangement(s) in other individuals classified as Niemann-Pick disease Type C remain to be established.

II. CLINICAL INVESTIGATIONS OF NEUROGENETIC DISEASES

Important contributions have been made by the Section on Clinical Investigations and Therapeutics concerning the identification of novel phenotypic presentations of Tay-Sachs disease, Menkes disease, biopterin deficiency syndrome, a new class of lipid storage disorders associated with ataxia, supranuclear ophthalmoplegia, dementia, and mild hepatosplenomegaly, and a novel form of glycerol kinase deficiency. Rare phenotypes of established genetic disorders have been documented. A large number of diagnoses have been made for physicians in other Institutes and from outside of NIH. Novel diagnostic tests have been developed using urine samples and the reliability of the prenatal diagnosis of genetic disorders by chorionic villus biopsy specimens has been established.

III. MODELS OF HUMAN LYSOSOMAL STORAGE DISORDERS

A. Pharmacological model of Gaucher's disease.

A tissue culture model of Gaucher's disease has been developed with rat peritoneal macrophages using conduritol-B-epoxide, a highly potent inhibitor of glucocerebrosidase. This system is expected to be useful for investigations designed to improve the delivery of glucocerebrosidase to macrophage storage cells. This investigation should assist in the development of effective enzyme replacement therapy for patients with Gaucher's disease.

B. Spontaneous murine model of Niemann-Pick disease Type C.

Information concerning the biochemical defect of cholesterol esterification established in this model resulted in the demonstration of a similar lesion in humans. Further work indicated that diagnostic tests based on this finding permit the identification of homozygous and heterozygous mice. Extrapolation of this procedure should lead to the development of similar tests for Niemann-Pick C patiénts and carriers. Experiments performed with tissues from these mice provide a unique opportunity to identify the specific protein involved in the murine and possibly the human [illegible]nditions.

C. Pharmacological model of Hurler's disease.

We discovered that the administration of suramin induces enzymatic and pathologic changes in experimental animals similar to those seen in mucopolysaccharidosis Type II (Hunter syndrome) in humans. The time course of the resolution of mucopolysaccharide accumulation and reversal of pathologic manifestations has been investigated. These studies have important implications concerning the reversibility of tissue damage in human mucopolysaccharide storage disorders. These studies are especially relevant since suramin is currently under investigation as a therapeutic agent for acquired immunodeficiency syndrome (AIDS).

D. Canine model of Hurler's disease.

The Branch has been involved in a collaborative study with investigators at the University of Tennessee who have established a colony of Plott hounds with a spontaneous mutation closely resembling mucopolysaccharidosis Type I (Hurler syndrome) in humans. Bone marrow transplantations have been performed on affected animals and the biochemical responses to this therapeutic approach are being monitored by DMNB. Acquisition of this information is particularly important for decisions regarding of bone marrow transplantation for patients with mucopolysaccharidoses and other metabolic disorders.

IV. STUDIES ON THE STRUCTURE AND CELL BIOLOGY OF LYSOSOMAL ENZYMES

Comprehensive examinations of the amino acid sequence, type and location of carbohydrate attachment, analysis of the active sites of these enzymes, and the subcellular sites of synthesis and the processing of these proteins have been carried out. These experiments have provided a wealth of information concerning events involved in the translocation of enzymes from their nascent state, the amino acid sequence of the leader polypeptide required for this process for glucocerebrosidase, and the type and composition of oligosaccharide side chains and the modifications that take place with maturation of the enzymes. A major discovery is that discrete mutations have occurred in the enzyme glucocerebrosidase in patients with various clinical presentations of Gaucher's disease. Thus, the altered enzyme in patients with neurological damage cannot reach lysosomes, the normal localization and site of action of such enzymes. On the other hand, the catalytically compromised enzyme in patients without CNS involvement does reach the lysosomes, but because of the mutation in the genetic code and hence in the amino acid sequence of the enzyme, it is less active than normally.

Other fundamental work includes the purification of α-iduronidase, the enzyme involved in Hurler syndrome, and sphingomyelinase, the enzyme that is lacking in patients with Types A and B Niemann-Pick disease. Preparation of antibodies to these enzymes should lead to discoveries of protein polymorphisms in the respective disorders and an understanding of the basis of the various clinical phenotypes that occur in these diseases similar to the techniques described in previous annual reports for discrimination of the various phenotypes of Gaucher's disease.

V. MOLECULAR GENETICS OF [illegible]YSOSOMAL STORAGE DISORDERS

The gene for glucocerebrosidase has been cloned by investigators in the section on Molecular and Medical Genetics. This is a major accomplishment concerning (1) the acquisition of knowledge of the molecular pathology in Gaucher's disease; (2) the possibility of producing glucocerebrosidase by recombinant DNA technology, (3) the development of new diagnostic procedures involving DNA restriction fragment length polymorphisms in patients and carriers of this disorder, (4) accurate gene mapping, and (5) potential therapeutic applications including considerations of gene engineering or replacement. To this end, retroviral vectors and appropriate test systems have been developed to establish the feasibility of transfer of the glucocerebrosidase gene. Furthermore, since the enzymes involved in Niemann-Pick disease and Hurler's diseases have now been purified, it is confidently expected that the genes for these enzymes will soon be isolated. These developments will permit examination of the chromosomal localization, identification of variations in the genes in patients, development of novel diagnostic tests, and comprehensive studies of the factors involved in the expression of these genes.

VI. ENZYME REPLACEMENT THERAPY FOR SPHINGOLIPID STORAGE DISORDERS

The structures of the N-asparagine linked oligosaccharides of glucocerebrosidase, the enzyme involved in Gaucher's disease, have been determined in detail. Procedures have been developed to modify and obtain large amounts of this enzyme specifically targeted to cells of the monocyte/macrophage system where the accumulating glucocerebroside is stored in patients with this disorder. A clinical trial of enzyme replacement using the modified enzyme has been initiated.

VII. MEMBRANE RECEPTORS FOR PHYSIOLOGICAL AND ENVIRONMENTAL SIGNALS

A. Role of Gangliosides as Signal Recognition Molecules.

1. Studies with fluorescent gangliosides.

Novel techniques developed in the Section on Membrane Biochemistry provide additional support for the concept that gangliosides are specific receptors for bacterial toxins and viruses. Fluorescent derivatives of ganglioside G_{M1} were synthesized and shown to be as effective as native G_{M1} as receptors for cholera toxin. The tagged gangliosides have been especially useful for subcellular localization of these sphingolipids and for investigating their interaction with other physiologically important glycoconjugates such as fibronectin.

2. Involvement of ganglioside(s) in mitogenic phenomena.

It has been shown that the B subunit of cholera toxin which binds to and cross-links several molecules of ganglioside G_{M1} on the surface of cells can elicit a proliferative stimulus in rat thymocytes. This response does not involve the activation of adenylate cyclase. Ganglioside aggregation caused by multivalent binding with the B subunit of cholera toxin is followed by an increase of Ca^{++} uptake in these cells. It is believed that the mitogenic effect is a consequence of an interaction of gangliosides with ion channels in the thymocytes and modulation of the physiological activity of these channels.

B. Regulation of Hormone-Responsive Adenylate Cyclase.

1. Desensitization of cells by human chorionic gonadotropin (hCG)

Experiments with a clonal line of murine Leydig tumor cells developed in the Membrane Biochemistry Section has provided insight into the mechanism of the loss of hCG-sensitive adenylate cyclase activity that occurs after exposure of these cells to hCG. This desensitization seems to be different from that caused by β-adrenergic agonists in that there is no loss of surface hCG-receptors or change in their affinity for hCG. Additional studies indicate that the hCG-receptor becomes phosphorylated on exposure to this hormone and this modification may be an important aspect of desensitization.

2. Desensitization of adenylate cyclase by phorbol esters.

Phorbol esters are widely used by cell biologists because of their tumor-promoting effects. One of the most frequently studied compounds is 12-O-tetradecanoylphorbol-13-acetate (TPA) which is a potent activator of protein kinase C. TPA causes desensitization of cells to β-adrenergic agonists by a mechansim distinct from that mediated by the agonists themselves. In contrast with the response to β-agonists which is characterized by a reduction in receptor activity, TPA treated cells had normal β-receptor activity. Both TPA and agonists cause a redistribution of β-receptors from the plasma membrane to a membrane fraction devoid of adenylate cyclase activity. Blocking receptor redistribution prevents TPA-, but not agonist-mediated desensitization. The redistribution of receptors, and hence segregation from the regulatory component of adenylate cyclase, may be the result of phosphorylation of the receptor catalyzed by protein kinase C. The desensitization mediated by β-agonists may also involve phosphorylation of the receptor, but at a different site resulting in alteration of activity and segregation. These investigations provide insight into the regulation of adenylate cyclase activity which mediates the physiological effects of many hormones and neurotransmitters.

VIII. DEMYELINATING DISORDERS

A. Myelin-associated Glycoprotein (MAG) and Glycolipid Antibodies in Autoimmune Neuropathies

A considerable number of patients have been identified who have combined motor and sensory peripheral neuropathy along with a monoclonal immunoglobulin M (IgM) in their sera that reacts with a highly antigenic epitope in the carbohydrate portion of MAG. This situation is becoming an increasingly important aspect of clinical neurology. The Section on Myelin and Brain Development has received numerous samples of serum from clinics throughout the world for testing for anti-MAG antibodies. Most of the patients' sera react with MAG and with two smaller glycoproteins and a sphingoglycolipid in peripheral nerve that share this antigenic determinant. Although MAG is present in both CNS and PNS mmyelin, the small glycoproteins and the sphingolipid that react with patients' IgM are localized exclusively in the PNS. The structure of the antigenic sphingolipid has been established in collaboration with investigators at the E.K.S. Center for Mental Retardation in Waltham, MA. It is a novel sulfated, glucuronic acid-containing sphingoglycolipid whose components and anomeric glycosidic linkages have been determined.

Some patients with IgM paraproteinemia and peripheral neuropathy have monoclonal antibodies directed against glycolipids in the nervous system but not MAG. Since all of the anti-MAG paraproteins also react with the glucuronic acid-containing sphingolipid in peripheral nerve, glycolipid antigens appear to be common in patients with paraproteinemic neuropathies and may be important in the pathogenesis of autoimmune peripheral neuropathies. This discovery may provide an important basis for investigating the etiology of certain demyelinating diseases of the central nervous system.

B. Shared Antigenicity between MAG and Glycoconjugates in Other Tissues

Experiments revealed that the monoclonal antibody HNK-1 (anti-Leu 7) raised against human lymphoma cells also reacts with a carbohydrate determinant in MAG. This antigen is present in a subset of human lymphocytes including natural killer cells and some suppressor cells. This duality of distribution of a common antigen between the immune system and oligodendrocytes has long been sought in autoimmune demyelinating diseases. This correlation is strengthened by the finding that the carbohydrate epitope recognized by HNK-1 and monoclonal anti-MAG antibodies are also present on certain tumors of neuroectodermal origin including melanoma and small cell lung carcinoma. Antigens of this type may play an important role in the pathogenesis of paraneoplastic neuropathies.

C. Potential Roles for MAG in Ontogenesis and in the Pathogenesis of Multiple Sclerosis.

Another important observation made during the past year is the finding that an antibody that reacts with the glycoprotein called the neural cell adhesion molecule (N-CAM) and a possibly related glycoprotein called the L1 adhesion molecule reacts with a carbohydrate epitope in MAG. Antibodies raised against MAG, the IgM paraproteins from patients with peripheral neuropathy, and HNK-1 react with N-CAM. These results indicate that several "adhesion molecules" which are believed to be important in tissue organization and differention share a carbohydrate determinant or determinants with MAG. The identification of this epitope is under investigation. The localization of MAG in the periaxonal membrane of oligodendrocytes and Schwann cells and its absence from compact myelin is consistent with a critical role for MAG in myelinogenesis and in the maintenance of the myelin sheath. MAG is cleaved by a proteolytic enzyme to a derivative protein (dMAG) that is 10,000 daltons smaller than MAG. The activity of this enzyme is increased over normal in brain tissue from patients with multiple sclerosis. It is believed that the cytoplasmic portion of MAG is cleaved by this process. The cytoplasmic domain of MAG may interact with actin in the myelin sheath. When converted to dMAG, this glycoprotein is easily released from myelin membranes, possibly because it is no longer anchored to actin. This observation is consistent with the finding that only dMAG is present in the CSF. The importance of this phenomenon to the pathology of multiple sclerosis is under investigation.

CONTRACT NARRATIVE

Developmental and Metabolic Neurology Branch
Intramural Research Program, NINCDS
October 1, 1984 through September 30, 1985

Contractor: GENZYME CORPORATION, BOSTON, MA. (N01-NS-3-2346)

Title: Preparation of Ceramidetrihexosidase from Human Placental Tissue

Contractor's Project Director: Henry E. Blair

Current Annual Level of Support: $104,005

Objectives: To isolate human placental ceramidetrihexosidase in sufficient purity and quantity for use in enzyme replacement trials in patients with Fabry's disease.

Major Findings: A procedure has been developed for the large-scale purification of human placental ceramidetrihexosidase in sufficient purity and specific catalytic activity so that it can be safely administered to patients with Fabry's disease. The contractor developed a procedure to remove pyrogen(s) that previously prevented administration of large quantities of ceramidetrihexosidase to patients. We have begun enzyme replacement trials with this pyrogen-free preparation.

Significance to Biomedical Research and to the Program of the Institute: A principal mission of the institute is to develop effective therapy to treat human diseases. If salutary clinical results can be obtained, an extraordinary milestone will have been accomplished regarding this type of a human genetic disease.

Proposed Course of the Contract: We are reinitiating enzyme replacement therapy in patients with Fabry's disease. We shall examine the effectiveness of the enzyme in patients with regard to clearance of accumulated ceramidetrihexoside in the liver and in the blood and monitor their clinical responses to this potential therapeutic agent.

CONTRACT NARRATIVE

Developmental and Metabolic Neurology Branch
Intramural Research Program, NINCDS
October 1, 1984 through September 30, 1985

Contractor: WEIZMANN INSTITUTE OF SCIENCE (NO1-NS-2349), Rehovot, Israel

Title: Production of Radiolabeled Glycolipids and Other Sphingolipid Derivatives

Contractor's Project Director: David Shapiro, Ph.D.

Current Annual Level of Support: $76,133

Objectives: To prepare glucocerebroside, sphingomyelin, and ceramidetrihexoside labeled with ^{14}C in critical portions of the molecule for diagnostic tests fro Gaucher's disease, Niemann-Pick disease, and Fabry's disease.

Major Findings: The principal investigator is a world-recognized expert in the chemical synthesis of sphingolipids. He has developed procedures to incorporate radioactive carbon-14 into specific portions of sphingolipid molecules. These compounds are used to diagnose patients with the sphingolipid storage disorders listed above, to identify heterozygous carriers of these conditions, to diagnose these disorders prenatally, and to monitor enzyme isolation procedures for glucocerebrosidase, sphingomyelinase, and ceramidetrihexosidase.

Significance to Biomedical Research and to the Program of the Institute: The ability to diagnose patients, identify heterozygotes, and monitor pregnancies at risk for sphingolipid storage disorders represents major contributions to the control of the incidence of these diseases. These procedures are in wide use at the present time.

Proposed Course of the Contract: The contractor will provide radioactive sphingolipids for enzyme purification procedures. He will also develop analogues of sphingolipids for the development of animal models of the human disorders. He will also prepare specific sphingolipid derivatives for use as ligands in affinity column chromatography to expedite and improve the isolation of sphingolipid hydrolases.

CONTRACT NARRATIVE

Developmental and Metabolic Neurology Branch
Intramural Research Program, NINCDS
October 1, 1984 through September 30, 1985

Contractor: GENZYME CORPORATION, BOSTON, MA. (NO1-NS-2351)

Title: Preparation of Glucocerebrosidase from Human Placental Tissue

Contractor's Project Director: Henry E. Blair

Current Annual Level of Support: $409,094

Objectives: To isolate human placental glucocerebrosidase in sufficient purity and quantity for use in enzyme replacement trial in patients with Gaucher's disease.

Major Findings: A procedure has been developed for the large-scale purification of human placental glucocerebrosidase in sufficient purity and specific catalytic activity so that it can be safely administered to patients with Gaucher's disease. The intravenous infusion of this enzyme appears to have retarded the progression of enlargement of the spleen and liver in patients with this disorder, stabilized their blood platelet count, and caused an improvement in the general health and growth patterns in some of the recipients.

Significance to Biomedical Research and to the Program of the Institute: A principal mission of the Institute is to develop effective therapy to treat human diseases. If the results indicated in the preceding paragraph can be confirmed and extended, an unprecedented feat will have been accomplished regarding human genetic diseases.

Proposed Course of the Contract: We have developed means to target the enzyme to the specific cells in which toxic quantities of lipid accumulate. When a sufficient quantity of the modified enzyme is available, we shall examine its efficiency in patients. We shall also continue to attempt to develop methods to deliver the enzyme to the central nervous system for the treatment of patients with the neuropathic forms of the disorder.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 00706-26 DMN

PERIOD COVERED

September 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Inborn Errors of Metabolism of Diverse Etiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

John A. Barranger, M. D., Ph.D.
Associate Chief, Developmental and Metabolic Neurology Branch, IRP, NINCDS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Clinical Investigations and Therapeutics/Molecular and Medical Genetics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.3	3.1	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The above project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 00815-25 DMN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Metabolism of Complex Lipids of Nervous Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. O. Brady, Chief	DMN	NINCDS
OTHERS:	P. G. Pentchev, Biochemist	DMN	NINCDS
	A. E. Gal, Organic Chemist	DMN	NINCDS
	T. Tokoro, Visiting Fellow	DMN	NINCDS
	J. M. Quirk, Biochemist	DMN	NINCDS
	M. Comly, Biologist	DMN	NINCDS
	H. S. Kruth, Senior Investigator	EA, IR	NHLBI

COOPERATING UNITS *(if any)*
Laboratory of Experimental Atherosclerosis, NHLBI

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Enzymology and Genetics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7	6	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
☒ (b) Human tissues
☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. The knowledge gained in our identification of the metabolic abnormality in the mutant strain of BALB/c mice as an inability to esterify exogenous cholesterol has been extended to a comprehensive investigation of patients with Type C Niemann-Pick disease. By far the majority of these individuals exhibit the profound deficiency of cholesterol esterification thus demonstrating the nature of the metabolic defect in this group of patients. This discovery paves the way for the development of tests for genetic counseling and eventual therapy for this human disorder of metabolism.

2. Other work has centered on the use of non-metabolizable analogues of glucocerebroside to examine the intercellular disposition and gastrointestinal excretion of this lipid which accumulates in Gaucher's disease. The results obtained in these investigations will be used to develop strategies for the treatment of patients with this disorder in addition to enzyme replacement.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 01309-20 DMN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Biosynthesis and Function of Glycosphingolipids and Other Glycoconjugates

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
PI: P. H. Fishman, Ph.D., Chief, Membrane Biochem. Section, DMN, NINCDS
OTHER: S. Spiegel, Ph.D., Visiting Fellow, DMN, NINCDS
G. Matyas, Ph.D., Staff Fellow, DMN, NINCDS
C. Frexias, M. S., Chemist, DMN, NINCDS
R. O. Brady, M.D., Branch Chief, DMN, NINCDS

COOPERATING UNITS (if any)
Laboratory of Cellular Metabolism, NHLBI
Laboratory of Kidney and Electrolyte Metabolism, NHLBI
Laboratory of Molecular Biology, NCI
Clinical Neuroscience Br., NIMH

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Membrane Biochemistry Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.9	1.9	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Gangliosides appear to be important recognition molecules on the cell surface and have been implicated as receptors for certain bacterial toxins and viruses. Little is known, however, about the normal physiological role(s) of these plasma membrane components. We have developed several approaches and model systems to address this issue. Fluorescent derivatives of ganglioside GM1 containing either rhodamine or Lucifer yellow CH were synthesized and shown to be as effective as native GM1 as receptors for cholera toxin. The fluorescent gangliosides were inserted into the plasma membrane of mouse and rat thymocytes and underwent capping when the cells were exposed to cholera toxin or anti-rhodamine antibodies, which are both multivalent. Cholera toxin also induced patching and capping of endogenous GM1 on the thymocyte surface. Exposure of rat thymocytes to the B or binding subunit of the toxin resulted in a proliferative response as measured by increased DNA synthesis. Even at 25 ng/ml, the B subunit was effective as a mitogen. Prior incubation of the B subunit with anticholera toxin antibodies blocked both its binding to and stimulation of the cells. The B subunit was shown to be free of any adenylate cyclase-activating A subunit; and cyclic AMP inhibited mitogenesis. Thus, binding of several molecules of GM1 on the thymocyte surface by the B subunit leads to the transduction across the plasma membrane of a mitogenic signal.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 01457-19 DMN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
The Chemical Synthesis of Radioactive Sphingolipids

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. E. Gal, Chief, Neurochemical Methodology	DMN	NINCDS
OTHER:	Patricia J. Voorstad, Chemist	DMN	NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Neurochemical Methodology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
☐ (b) Human tissues
☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Sphingolipids containing radioactive isotopes were synthesized and used for metabolic studies and as diagnostic tools in sphingolipidoses. ^{14}C and ^{3}H labels were introduced by synthetic and semi-synthetic techniques, gas exposure, and a new approach: functional group exchange. These techniques were used for the syntheses of radioactive enantimorphic derivatives of sphingolipids. These products are not metabolizable. Experimentation with these in animals creates "animal models" for metabolic diseases and opens new areas for biomedical studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 NS 01808-16 DMN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Glycoproteins of Myelin in Development and Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Richard H. Quarles	Section Chief	DMNB, NINCDS
OTHERS:	Antonio Noronha	Guest Researcher	DMNB, NINCDS
	Amjad Ilyas	Visiting Fellow	DMNB, NINCDS
	Daniel O'Shannessy	Visiting Fellow	DMNB, NINCDS
	Katsuhiko Yanagisawa	Visiting Fellow	DMNB, NINCDS
	Hugh Willison	Visiting Fellow	DMNB, NINCDS
	Johanna Moller	Visiting Fellow	DMNB, NINCDS
	Roscoe O. Brady	Branch Chief	DMNB, NINCDS

COOPERATING UNITS (if any)
Children's Hospital Medical Center, Boston, MA; E. K. Shriver Center for Mental Retardation, Waltham, MA; Department of Neurology, Johns Hopkins Univ., Baltimore, MD.; Laboratory of Molecular Genetics, NINCDS; Neuroimmunology Branch, NINCDS

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Section on Myelin and Brain Development

INSTITUTE AND LOCATION
NINCDS, Park Building, Rm. 425, NINCDS, NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.9	7.1	1.8

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The myelin-associated glycoprotein (MAG) is localized in the periaxonal part of PNS and CNS myelin sheaths where it appears to be involved in glia-axon interactions. The amount of MAG in Jimpy mice with a severe hypomyelination is reduced to 5% of the control level, but its apparent molecular weight is not higher than normal as had previously been described in Quaking and Trembler mice. Several types of monoclonal antibodies have now been identified that react with related carbohydrate epitopes in MAG and other glycoconjugates. These include: 1. several that were generated by MBDS in mice immunized with MAG; 2. HNK-1, that reacts with a subset of human lymphocytes; 3. 10C5, an antibody reacting with glycoproteins in melanoma, small cell lung carcinoma and other tumors of neuroectodermal origin; 4. L2, an antibody reacting with several proteins involved in cell-cell interactions including neural cell adhesion molecule (N-CAM); and 5. human monoclonal IgM in paraproteinemia associated with neuropathy. In addition to MAG, each of these antibodies reacts with glycolipids and 20 to 26K dalton glycoproteins that are specific for the PNS. The principal glycolipid reacting with these antibodies is an unusual sulfated, glucoronic acid-containing sphingoglycolipid that had not previously been described. The carbohydrate epitopes in MAG and other glycoconjugates of nervous tissue that are shared with the immune system and various tumors of neuroectodermal origin may be relevant to demyelinating diseases. A number of patients with IgM paraproteinemia associated with neuropathy have been identified in which the IgM does not react with MAG but does react with other glycolipids, indicating that glycolipid antigens are frequent in neuropathy associated with IgM gammopathy. Relatively high levels of a proteolytic derivative of MAG, dMAG are present in all cerebrospinal fluids, but the amount does not correlate with active demyelination. A low level of anti-MAG antibodies was detected in the cerebrospinal fluid of multiple sclerosis patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02162-11 DMN

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Synthesis of Compounds Analogous to Glycolipids

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Andrew E. Gal, Ph.D., Chief, Neurochemical Methodology Section DMN, NINCDS

OTHER: Patricia J. Voorstad, Chemist, Neurochemical Methology Section DMN, NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Neurochemical Methodology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	0.7	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Work was continued on the syntheses of glycolipid analogues of sphingolipids that yield a chromogenic moiety on enzymatic hydrolysis. These compounds are used for the diagnosis and studies of Niemann-Pick, Gaucher's and Krabbe's disease.

Conduritol B epoxide, a saccharide that strongly inhibits β-glucosidases, was synthesized by a method developed by this section that provides the product in greater yield than previously available and permits the preparation of this compound containing a tracer with extraordinarily high specific radioactivity. Administration of conduritol B-epoxide to animals produces a syndrome that resembles Gaucher's disease in humans by inhibiting the enzyme glucocerebrosidase. Radioactive conduritol B-epoxide reacts with the active site of glucocerebrosidase isolated from normal human tissues and from patients with Gaucher's disease. This use of the radioactive conduritol β-epoxide will materially accelerate the identification of the amino acid substitutions (or deletions) that occur in the glucocerebrosidase moledule in patients with Gaucher's disease. Also fluorescent and cytotoxic substrates were prepared which can be used for the separation of affected and normal cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02163-11 DMN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Development of Analytical Methods for the Use of Research of Sphingolipidoses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
PI: Andrew E. Gal, Chief, Neurochemical Methodology Section DMN, NINCDS
OTHER: Patricia J. Voorstad, Chemist DMN, NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Neurochemical Methodology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

New analytical techniques were developed and used in enzymatic research and in clinical investigations of lipidoses. The lipid content in human tissues, the diagnosis of lipid storage diseases by gas, thin-layer chromatography and other techniques were studied at the microgram level. The techniques we developed previously were improved, modified and used in connection with ongoing projects related to lipidoses in our laboratories and also as joint projects with outside groups. Numerous analytical studies were undertaken by using these techniques.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01NS 02366-07 DMN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Regulation of Hormone-Responsive Adenylate Cyclase

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P. H. Fishman, Chief, Membrane Biochemistry Section, DMN, NINCDS
OTHER: R. V. Rebois, Ph.D., Senior Staff Fellow, DMN, NINCDS
S. Kassis, Ph.D., Visiting Associate, DMN, NINCDS
M. Schramm, Ph.D., Visiting Scientist, IRP, NINCDS
R. M. Bradley, B. S., Chemist, DMN, NINCDS
M. A. Sullivan, B.S., Biologist, DMN, NINCDS

COOPERATING UNITS (if any)
Laboratory of Clinical Science, NIMH

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Membrane Biochemistry Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.2	3.2	2.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have been investigating the mechanism of densensitization of hormone-stimulated adenylate cyclase in mammalian cells. Exposure of a clonal line of murine Leydig tumor cells to human chorionic gonadotropin (hCG) resulted in a rapid attenuation of hCG-stimulated adenylate cyclase activity. This desensitiation was both time and dose dependent and occurred without any loss of cell surface hCG-receptors or any change in their affinity for hCG. Desensitization did not occur when the cells were exposed to hCG at 0°C but did occur when protein synthesis was blocked by cycloheximide. Desensitization of hCG-stimulated adenylate cyclase also occured in cells exposed to phorbol esters which activate protein kinase C. There was increased incorporation of ^{32}P into the hCG-receptor of cells desensitized by either hCG or phorbol esters. Thus, receptor phosphorylation may be the basis for desensitization. Both agonists and phorbol esters caused desensitization of the adrenergic-stimulated adenylate cyclase in rat glioma C6 cells but by distinct mechanisms. Either treatment caused a redistribution of β-receptors from the plasma membrane to a lighter density membrane fraction which was devoid of adenylate cyclase activity. Prior treatment of the cells with concanavalin A prevented this shift in receptors. The lectin pretreatment also prevented the phorbol ester-, but not the agonist-mediated desensitization. Finally, β-receptor functional activity was reduced in cells desensitized by it physical separation from adenylate cyclase. In contrast, phorbol ester-mediated desensitization involves only the physical separation of the receptor from adenylate cyclase. Receptor phosphorylation may be involved in both types of desensitization but at different sites on the receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02433-06 DMN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Models of Lysosomal Storage Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John A. Barranger, M.D., Ph.D
Associate Chief, DMNB, IRP, NINCDS

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Clinical Investigations and Therapeutics/Molecular and Medical Genetics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS: 2.5	**PROFESSIONAL:** 2.0	**OTHER:** 0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The above project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	**PROJECT NUMBER** Z01 NS 02434-06 DMN

PERIOD COVERED October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of Lysosomal Function: Receptor-Mediated Pinocytosis of Lysosomal Enzymes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John A. Barranger, M.D., Ph.D., Associate Chief
DMN, IRP, NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Clinical Investigations and Therapeutics/Molecular and Medical Genetics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
☒ (b) Human tissues
☐ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The above project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	**PROJECT NUMBER** Z01 NS 02435-06 DMN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies on the Mechanism of Pathogenesis of the Mucopolysaccharidoses.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

George Constantopoulos, Ph.D., Research Biochemist, DMNB, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Enzymology and Genetics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The mucopolysaccharidoses (MPS) are a group of hereditary diseases characterized by defective metabolism of glycosaminoglycans (GAG). The disorders are usually associated with severe dysfunction of the nervous system as well as of liver, spleen, heart, bone, and other tissues. Objective of this project is the study of mechanism of pathogenesis of these diseases with emphasis on brain involvement and mental retardation. We are using a comparative approach. For this purpose we study the changes, in GAG, sphingolipids, and pertinent lysosomal enzymes in tissues of patients with various types of MPS and we make correlation in terms of clinical and ultrastructural findings. Our laboratory contributed significantly in understanding the chemical pathology and in particular the neurochemistry of MPS IH, MPS IS, MPS II, MPS III A and MPS III B. To complement the studies with human subjects, a drug (suramin) induced animal model of MPS has been developed and a canine model, (natural), of MPS I is being studied. Both animal models may prove useful for understanding the pathogenesis of MPS and in the development and assessment of therapeutic trials by enzyme replacement.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02453-05 DMN

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gaucher's Disease: Biochemical and Clinical Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

John A. Barranger, M.D., Ph.D., Assoc. Chief, DMNB, IRP, NINCDS;
Others: Norman Barton, M.D., Ph.D., John Fink, M.D., Warren Cohen, M.D., Ph.D., Gary Murray, Ph.D., Lori Hampton, Carol Moore, Susan Sorrell, Gregory Zirzow, Mark Garfield, Beverly Smith, Henry O'Connell, and Pijush Das, Ph.D., CITS, DMNB, NINCDS; Edward Ginns, M.D., Ph.D., Brian Martin, Ph.D., Shoji Tsuji, M.D., PhD., MMGS, DMNB, NINCDS; Drs. H. Mankin and S. Doppelt, MGH, Boston, MA; Dr. J. Tager, Univ. of Amsterdam; Dr. A. Erickson, Rockefeller Univ., NY; Dr. Arnold Reuser, Erasmus Univ., The Netherlands.

COOPERATING UNITS (if any)

Dept. of Orthopaedic Surgery, Massachusetts General Hospital, Boston, MA; Dept. of Biochem., Univ. of Amsterdam, The Netherlands; Dept. of Genetics, Erasmus Univ., The Netherlands; Dept. of Cell Biology, Rockefeller Institute, New York, NY.

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Clinical Investigations & Therapeutics Sect./Molecular & Medical Genetics Sect.

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects
  ☒ (a1) Minors
  ☐ (a2) Interviews
☒ (b) Human tissues
☐ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Conventional or novel therapy for Gaucher's disease depends upon broad clinical and basic scientific knowledge of the disorder. Many patients have been studied and important complications identified. In terms of diagnosis, methods have been developed which permit identification of cases using only a urine sample. Diagnosis of different phenotypes using a monoclonal antibody permits identification of neurologically affected cases presymptomatically. A disorder of vitamin D metabolism affecting calcium homeostasis has been described and regimens of vitamin D and calcium supplementation are being evaluated. Basic research work on glucocerebrosidase has generated a variety of new and complementary projects which address the biochemistry, cell biology, and molecular genetics of the enzyme as a part of more far-reaching studies. Glucocerebrosidase serves as a model for these studies of lysosomal enzymes and proteins. The results of this coordinated approach have revealed the structure, biosynthesis, rates of synthesis and degradation, lysosomal routing, lectin binding, and cellular uptake of the enzyme. The alteration of some of these processes have been described for the several different mutations of the gene resulting in different phenotypes of the disease. This information provides substantive new data from which the approach of enzyme replacement is perfected. A clinical trial incorporating these advances is currently underway. Other projects have resulted in the isolation, expression, and transfer of the gene for glucocerebrosidase and are leading to the consideration of gene transfer for Gaucher's disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02529-04 DMN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Development of Enzymes That Inactivate Neurotoxic Agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Roscoe O. Brady, Chief	DMN	NINCDS
OTHER:	J. M. Poston	LB	NHLBI
	A. E. Gal	DMN	NINCDS

COOPERATING UNITS (if any)
Laboratory of Biochemistry, NHLBI

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Enzymology and Genetics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS: 0.2	PROFESSIONAL: 0.2	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An enzyme that degrades barbital has been identified and partially purified from extracts derived from a soil micro-organism. The requirements for maximal catalytic activity are being determined. We attempted to scale-up the production of this enzyme to examine its effectiveness in reversing the effects of lethal quantities of barbital in toxicological experiments with appropriate animals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS-02619-02 DMN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Oxidative Metabolism in Patients with Inherited Neurological Diseases and in Mycoplasmas.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
PI: George Constantopoulos, Ph., Research Biochemist, DMNB, NINCDS

COOPERATING UNITS (if any)
Institute for Medical Research, Camden, New Jersey
Surgical Neurology Branch, NINCDS

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Enzymology and Genetics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.3	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An increasing amount of evidence points to a possible defect in oxidative metabolism in patients with certain inherited neurological disorders. Thus, a defect in the pyruvate oxidation system has been shown in some patients with lactic acidemia and diffuse neurologic disease, of the mitochondrial malic enzyme in patients with Friedreich's ataxia, and a partial deficiency of glutamate dehydrogenase in some patients with olivopontocerebellar degeneration. However, there is much controversy about the exact enzymatic defect(s). The objective of this project is the elucidation of the defect in some of these patients or in skin fibroblasts derived from such patients. For this purpose we are assaying a number of mitochondrial and non-mitochondrial enzymes in fibroblasts or leukocytes and we have initiated electron microscopic studies of the mitochondria. We became interested in the oxidative metabolism of mycoplasmas because mycoplasma contamination of fibroblast cultures interfered with the assay of pyruvate dehydrogenase complex in these cells. The oxidative metabolism of mycoplasmas is poorly understood. Hopefully, the elucidation of the defect in these diseases will help in the diagnosis and therapeutic intervention in these patients. Knowledge of the physiology of mycoplasmas may help in understanding the pathogenicity of these organisms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02648-01 DMN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Biological Modification of Human Glioma Cells In Vitro

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: George Constantopoulos, Ph.D.,Research Chemist, DMNB, NINCDS
OTHER: Roscoe O. Brady, M.D., Chief DMNB, NINCDS
Paul L. Kornblith, Chief SNB, NINCDS

COOPERATING UNITS (if any)
Surgical Neurology Branch, NINCDS

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Enzymology and Genetics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Glycosaminoglycans (GAG) are polyanionic compounds, usually bound covalently to a protein core. They are a prominent component of the cell surface and are implicated in cell-cell interaction. Human glioma cells in tissue culture produce a much greater amount of hyaluronic acid (HA) than normal glial cells and they are shedding it in the media. Also, certain glioma cell lines are forming a hyaluronidase-sensitive "protective' coat that impairs their ability to elicit a allogeneic CTL response. We are using glucocorticoids (Dexamethasone) or dimethylsulfoxide (DMSO) to inhibit the biosynthesis of HA and other GAG in cultured glioma cells with the objective to make them more susceptible to immune response and/or chemotherapy. Since Dexamethasone can be administered to humans, such a modification may be useful in the management of patients with gliomas.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01NS02657-01 DMN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Molecular and Genetic Studies of Niemann-Pick Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Norman Barton, M.D., Ph.D., Clin. Investiga. & Therapeutics Sec., DMNB, NINCDS
Others: John A. Barranger, M.D., Ph.D., Assoc. Chief, DMNB, NINCDS; Katherine Oliver, Carol Moore, Gary J. Murray, Ph.D., Clinical Investigations & Therapeutics Sec., DMNB, NINCDS; Brian Martin, Ph.D. and Edward Ginns, M.D., Ph.D., Mol. & Medical Genetics Sec., DMNB, NINCDS; Dr. K. Sandhoff & Dr. G. Weitz, Univ. of Bonn, Germany Drs. J. Tager & A. Schram, Univ. of Amsterdam, The Netherlands.

COOPERATING UNITS (if any)
University of Amsterdam, Department of Biochemistry, The Netherlands
University of Bonn, Institute for Organische Chemie Und Biochemie, Germany

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Clinical Investigations & Therapeutics Sect./Molecular & Medical Genetics

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	2.6	0.2

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Niemann-Pick Disease is a progressively debilitating, neurogenetic disorder which is characterized biochemically by the accumulation of sphingomyelin in several tissues and organs in conjunction with deficiency of the lysosomal hydrolase, sphingomyelinase. Several ill-defined phenotypes of Niemann-Pick Disease have been reported in the clinical literature. Detailed description of these various phenotypes in terms of cellular pathochemistry and molecular genetics has not been accomplished to date. A major obstacle in this area has been the consistent absence of reproducible techniques for the isolation of homogeneous preparations of sphingomyelinase. Employing novel detergent and chromatography systems, we have successfully and reproducibly purified sphingomyelinase to homogeneity. The purified enzyme migrates with an apparent molecular weight of 52,000 daltons in SDS-polyacrylamide gels under both reducing and nonreducing conditions. Detailed kinetic analyses and determinations of the primary protein structure and carbohydrate composition are in progress as are efforts to develop and characterize monoclonal and polyclonal antibodies to the purified enzyme. Availability of well characterized antibodies will allow us to proceed immediately to cloning of the gene for sphingomyelinase. Characterization of the phenotypes of Niemann-Pick Disease in terms of protein polymorphisms and specific mutations at the DNA level will become a reality.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01NS02658-01 DMN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Sites of Carbohydrate Attachment in Lysosomal Enzymes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Brian M. Martin, Ph.D., Visiting Scientist, Molecular & Medical Genetics Section, DMNB, IRP, NINCDS
Others: John A. Barranger, M.D., Ph.D., Assoc. Chief, DMNB, NINCDS;
Denise Merkle-Lehman, and Gary Murray, Ph.D., Ph.D., Clin. Investigations & Therapeutics Section, DMNB, NINCDS;
Edward I. Ginns, M.D., Ph.D. and June Mayor, Molecular & Medical Genetics Section, DMNB, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Molecular and Medical Genetics Sect./Clin. Investigations & Therapeutics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.4	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Lysosomal hydrolases thus far purified contain oligosaccharide chains which are presumably involved in lysosomal translocation. In the case of glucocerebrosidase, we have shown that the three forms of the enzyme found in fibroblasts are reduced to a single form upon removal of the carbohydrate by endoglycosidase F. Similarly, the single higher molecular weight form isolated from placental tissue upon endoglycosidase F treatment apparently differs only by its carbohydrate content from the forms found in fibroblasts. The carbohydrate structure of the placental enzyme has been determined and the number of attachment sites was proposed to be four per molecule. All sites were suggested to be N-glycosylated asparagines. This is in good agreement with the four sites suggested by endoglycosidase H treatment of the high mannose form of glucocerebrosidase. The cDNA sequence for the gene for glucocerebrosidase, determined in our laboratory, identified five potential N-glycosylated asparagines.

In order to better understand the processing of lysosomal enzymes and define the exact number of carbohydrate sites in glucocerebrosidase, we have determined the amino acid sequence of the glycopeptides isolated from glucocerebrosidase. We have identified four glycopeptides, three from tryptic digests and one from cyanogen bromide. In each peptide, only one N-glycosylated asparagine was present. The fifth site, potentially present in glucocerebrosidase, was found to be a free asparagine. All four sites of carbohydrate attachment are the typical Asn(CHO)-X-Thr/Ser sequence. The sequences determined are: Asn(CHO)-Ala-Thr, Asn(CHO)-His-Thr, Asn(CHO)-Phe-Ser and Asn(CHO)-Ser-Thr. All the carbohydrate sites are located in the amino-terminal half of the molecule and may well play a role in both transport to the lysosome and localization in the membrane. Glycopeptides from α-galactosidase A, sphingomyelinase, and iduronidase are being studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01NS02659-01 DMN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Protein Structure of Lysosomal Enzymes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
Brian Martin, Ph.D., Visiting Scientist, Molecular & Medical Genetics Section, DMNB, IRP, NINCDS
Others: John A. Barranger, M.D., Ph.D., Assoc. Chief, DMNB, NINCDS
Denise Merkle-Lehman, Gary Murray, Ph.D., and Norman Barton, M.D., Ph.D., Clin. Investigations & Therapeutics Section, DMNB, NINCDS;
Edward I. Ginns, M.D., Ph.D., Molecular & Medical Genetics Section, DMNB, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Molecular & Medical Genetics Sect./Clinical Investigations & Therapeutics Sect.

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.4	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

As part of efforts to better define the biochemical defects underlying lysosomal storage disorders, we have undertaken studies on the primary structure of several lysosomal enzymes. Initial studies focused on glucocerebrosidase, the enzyme activity deficient in Gaucher's disease, as a prototype for these investigations.

In order to complete the amino acid sequence of human placental glucocerebrosidase, peptides were generated by both chemical (cyanogen bromide) and enzymatic (trypsin and V8 protease) cleavage. The peptides were separated by analytical high performance liquid chromatography using reverse-phase columns. The separation of the tryptic digest on the reverse-phase system provided a peptide map which will be useful for studying mutant glucocerebrosidases. The complete amino acid sequence of glucocerebrosidase was determined from the sequence of peptides obtained from the digests. Hence, it was possible to design and prepare oligonucleotide probes from regions of the amino acid sequence which were used to align and confirm the cDNA sequence of the gene cloned for glucocerebrosidase.

The secondary structure predicted from the amino acid sequence shows large areas of alpha-helix separated by beta-sheets and turns. Plots of hydropathy reveal alternating stretches of hydrophobic and hydrophilic amino acids throughout the primary structure. Of particular note is the correlation of several stretches of hydrophobicity in regions of α-helical structure.

The studies on the primary structure of glucocerebrosidase have revealed the presence of a single free sulfydryl and three disulfide bonds. With the identification of the disulfide bridges, we will construct a model of glucocerebrosidase both in terms of its enzyme activity and its membrane localization, utilizing the secondary structure predictions and hydropathy plots.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01NS02660-01 DMN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Analysis of the Active-Site of Glucocerebrosidase

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
Brian Martin, Ph.D., Visiting Scientist, Molecular & Medical Genetics Section, DMNB, IRP, NINCDS
Others: John A. Barranger, M.D., Ph.D., Assoc. Chief, DMNB, NINCDS;
Denise Merkle-Lehman and Gary Murray, Ph.D., Clinical Investigations and Therapeutics Section, DMNB, NINCDS;
Edward I. Ginns, M.D., Ph.D., Molecular & Medical Genetics Section, DMNB, NINCDS
Andrew Gal, Ph.D., DMNB, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Molecular and Medical Genetic Sect./Clinical Invest. & Therapeutics Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.4	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Glucocerebrosidase has greatly reduced activity in patients with Gaucher's disease. In order to better understand the structure-function relationship of this enzyme in both normal and Gaucher tissue, we have undertaken studies of the active-site of the human placental enzyme as the reference to which the mutant species may be compared. Conduritol-β-epoxide, a potent specific inhibitor of membrane bound glucocerebrosidase, has been used to probe the active-site of human placental glucocerebrosidase. While inhbition of activity occurred readily, inclusion of 0.1% taurocholate in the incubation buffer decreased the time of inactivation by 50%. Studies to locate the precise amino acid which reacts with the conduritol-β-epoxide are in progress using a radioactive form of the inhibitor. In this regard the tryptic peptide map generated during our amino acid sequence studies has been helpful.

Inactivation of the enzyme also occurred upon alkylation of a single sulfhydryl group by 4-vinyl pyridine (as judged by amino acid analysis). Inclusion of taurocholate, however, failed to enhance the inactivation. Although alkylation of the sulfhydryl by 4-vinyl pyridine inactivated the enzyme, there was no effect on activity when alkylation was attempted using either iodoacetate or iodoacetamide. One would postulate that the sulfhydryl was near the active-site but not involved in activity. The large bulky pyridine could prevent access of the substrate to the active-site. The identification of the free sulfhydryl is continuing as a part of the amino acid sequence studies of glucocerebrosidase.

In addition, we are investigating the effect of phospholipids and sphingolipid-activating protein (SAP-2) on the inactivation of glucocerebrosidase by conduritol-β-epoxide and vinyl-pyridine. Do either phospholipids or SAP-2 protect glucocerebrosidase from inactivation? These studies, in addition to defining the active-site, will provide insight into the alteration of catalytic activity of the mutant proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02661-01 DMN

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cell Biology and Biochemistry of Lysosomal Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Gary J. Murray, Ph.D., Visiting Associate, CITS, DMNB, NINCDS
Others: John A. Barranger, M.D., Ph.D., Assoc. Chief, DMNB, NINCDS;
Edward I. Ginns, M.D., Ph.D. & Brian Martin, Ph.D., MMGS, DMNB, NINCDS;
Susan Sorrell, Lori Hampton, Carol Moore, Lynn DeVaughn, Donna Huang,
Mark Garfield, Gregory Zirzow, & Pijush Das, Ph.D., CITS, DMNB, NINCDS;
Ann Erickson, Ph.D., Rockefeller Univ.; Joseph Tager, Ph.D., Univ. of
Amsterdam; and Arnold Reuser, Ph.D., Erasmus University.

COOPERATING UNITS (if any)

University of Amsterdam, Department of Biochemistry
Erasmus University, Department of Genetics
Rockefeller University

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Clinical Investigations and Therapeutics Sect./Molecular & Medical Genetics Sect.

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	5.7	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Macromolecules are synthesized and translocated to biomembranes in a specific fashion. Localization of structural proteins, receptors, transport proteins, and enzymes to plasma membrane or subcellular particles is an ordered process giving functionality to the membrane interface or lumen of the structure. Few of the chemical determinants responsible for this organization have been identified. The work focuses on the lysosome as one particle to which a number of proteins and glycoproteins are localized. Studies seek to define structural organization within the particle and list the properties of the lysosomal membrane and translocated protein responsible for that organization. Alteration of these normal processes in lysosomal storage diseases was examined. Study of the biosynthesis and translocation of glucocerebrosidase showed that the glycoprotein enzyme is synthesized on the rough endoplasmic reticulum (ER) and translocated to the lumen of the ER via a leader polypeptide of 2 k Da which interacts with a signal recognition particle (SRP). Cotranslationally, the polypeptide undergoes core glycosylation. This early glycosylated form is the precursor and is a high mannose glycoprotein which undergoes post-translational processing to a complex-type mature form of smaller molecular weight. The protein is neither phosphorylated in the oligosaccharide nor routed to the lysosome via the mannose-6-phosphate pathway. The precursor, intermediate and mature forms of the enzyme can be found as cross reactive material in cell extracts. This protein polymorphism allows the identification of different mutations in the gene for the enzyme which is responsible for different phenotypes of Gaucher's disease. Ultrastructural immunocytochemistry reveals that the protein does not appear in the lysosome of the neurologically affected variants whereas in the non-neurologically affected phenotypes the catalytically altered protein is present in normal amounts. Altered biosynthesis and translocation of mutant glucocerebrosidase has been defined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02662-01 DMN

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Engineering of Human Lysosomal Enzymes: Studies of Enzyme Replacement

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gary J. Murray, Ph.D., Clin. Investiga. & Therapeutics Sec., DMNB, NINCDS
Others: John A. Barranger, M.D., Ph.D., Assoc. Chief, DMNB, NINCDS;
Brian Martin, Ph.D. & Edward I. Ginns, M.D., Ph.D., MMGS, DMNB;
Mark Garfield, Susan Sorrell, Carol Moore, Gregory Zirzow, and
Henry O'Connell, CITS, DMNB, NINCDS.

COOPERATING UNITS *(if any)*

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Clinical Investigations & Therapeutics Sect./Molecular & Medical Genetics Sect.

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.3	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The lysosome, a specialized sub-cellular catabolic organelle, contains a variety of hydrolytic enzymes responsible for the degradation of a wide range of biomolecules. Many inherited disorders of lysosomal function occur as a result of mutations in the genes for these lysosomal acid hydrolases. Since the protein content of the lysosomes is determined by both intracellular and extracellular protein trafficking, it may be possible to target constitutive lysosomal proteins to the particle from an exogenous pool. By utilizing the normal biosynthetic and translocation mechanisms and the capacity for receptor mediated endocytosis, lysosomal enzymes may be replaced. Ideally, suitable engineering of the enzyme molecule permits binding to endocytic receptors (lectins) and high efficiency translocation to the lysosome where the enzyme maintains activity for a suitably long duration. Because lysosomal storage of glucocerebroside occurs only in tissue macrophages in Gaucher disease, glucocerebrosidase was chosen to serve as a prototype for the study of enzyme replacement. This protein for which the gene has now been isolated and characterized was purified to homogeneity and extensively characterized with respect to amino acid sequence, complete oligosaccharide structure, biosynthesis and translocation in order to evaluate its potential for these studies. Procedures were developed for the large scale enzymatic modification of the carbohydrate portion of glucocerebrosidase to take advantage of naturally occurring lectin receptors on the membranes of reticuloendothelial cells. By complete characterization of glucocerebrosidase with respect to carbohydrate structure and composition, it became possible to develop new analytical methods and adapt old ones to permit quantitative measurement of the extent of the modification of the oligosaccharide without loss of enzymatic activity. The efficiency of the delivery of glucocerebrosidase to reticuloendothelial cells in rats has been studied to predict the therapeutic potential of the treatment for Gaucher disease. In addition, cell culture models using animal and human macrophages have been developed for use in this evaluation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02663-01 DMN

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Organization of Human Glucocerebrosidase Gene

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Prabhakara V. Choudary, Senior Staff Fellow, MMGS, DMNB, NINCDS
Others: John A. Barranger, M.D., Ph.D., Assoc. Chief, DMNB, NINCDS;
Edward Ginns, M.D., Ph.D., Brian Martin, Ph.D., Barbara Stubblefield, Suzanne Winfield, Nancy Miller, Ph.D., Shoji Tsuji, M.D., Ph.D., June Mayor, Mary LaMarca, and Carl Lauter, MMGS, DMNB, NINCDS;
Gary Murray, Ph.D., CITS, DMNB, NINCDS.
Mia Horowitz, Ph.D., Weizmann Inst. of Science, Rehovot, Israel

COOPERATING UNITS (if any)

Weizmann Institute, Rehovot, Israel;
Genex Corporation, Gaithersburg, MD

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Molecular & Medical Genetics Sect./Clinical Investigations & Therapeutics Sect.

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.4	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Glucocerebrosidase in man is encoded by a gene of approximately 6.5 kb. We have isolated three full length copies of this gene, one from a HaeIII-AluI fetal genomic library and two from an EcoRI adult liver library in bacteriophage λ charon 4A. The 15 kb human genomic DNA segment containing this gene in all three clones has been characterized by restriction mapping. The 5' halves of the clones from the two different libraries show significant size heteromorphisms. We are in the process of analyzing the 5'ends of the genes, and the flanking sequences for putative eukaryotic promoter/enhancer elements, and the intron-exon relationships. Extensive characterization of these clones is in progress by D-loop analysis, restriction pattern analysis, and nucleotide sequence. The aim of these studies is to construct a physical map of the human glucocerebrosidase gene and identify the structural and regulatory elements in order to correlate the structural features with different functional domains of the glycoprotein. An understanding of the structural organization of glucocerebrosidase gene will be of value in identifying and defining the molecular nature of the genetic defect(s) leading to Gaucher's disease, in addition to elucidating the molecular mechanism(s) that are altered by the mutations in the gene. This will ultimately lead to a better understanding of the regulation of the glucocerebrosidase gene in man.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02664-01 DMN

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Studies of Neurogenetic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

John A. Barranger, M.D., Ph.D., Assoc. Chief, DMNB, NINCDS.

Others: Edward Ginns, M.D., Ph.D., Mol. & Med. Genetics Sect., DMNB, NINCDS; Warren Cohen, M.D., Norman Barton, M.D., Ph.D., Gary Murray, Ph.D., Susan Sorrell, Carol Moore, Bev Smith & Lori Hampton, CITS, DMNB, NINCDS; Joseph Tager, Ph.D. & Andre Schram, Ph.D., Univ. of Amsterdam; Genetics Fellows, Interinstitute Medical Genetics, CC, NIH; Frank King, M.D., & Hamal Ishak, M.D., Ph.D., A.F. Inst. of Path.; Henry Mankin, M.D., & Samuel Doppelt, M.D., MGH, Boston, MA; David Ullman, Ph.D., Veterans Hospital, MA.

COOPERATING UNITS (if any)

Interinstitute Medical Genetics Program, CC, NIH; Human Genetics Branch, NICHD; Lab. of Biochem., Univ. of Amsterdam, The Netherlands; Massachusetts Gen. Hosp.; Armed Forces Inst. of Pathol.; Veterans Hosp., GRECC, Waltham, MA.

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Clinical Investigations & Therapeutics Sect./Molecular & Medical Genetics Sect.

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.0	5.5	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The clinical study of neurogenetic diseases provides the context in which the goals of improved diagnosis and potential treatment modalities are identified. It both supports and stimulates the research necessary to achieve these objectives. Several new phenotypes have been recognized including a number of cases of the deficiency of hexosaminidase A presenting as motor neuron disease; glycerol kinase deficiency presenting as acidemia, stupor, and without mental retardation; biopterin deficiency presenting as familial dystonia; and a variety of cases of so-called Niemann-Pick disease, Type C. These later cases have permitted the demonstration that these phenotypes are not related to a defect in sphingomyelinase as previously believed. A number of rare phenotypes have also been identified including Tay-Sachs disease in a young non-Jewish child, 2 cases of juvenile Krabbe's disease, an unusual presentation of multiple sulfatase deficiency, an unusual case of San Filippo A disease, a case of arylsulfatase activator deficiency, a case of Menkes disease in a female infant, and several cases of acute neuronopathic Gaucher's disease. A large number of typical neurogenetic diseases have been confirmed by studies performed in this protocol. New diagnostic methods have been developed which permit the accurate diagnosis of Gaucher's and Niemann-Pick disease in a urine sample. Studies of chorionic villus samples (CVS) have allowed the prenatal diagnosis of a number of lysosomal storage diseases. We have developed an accurate method for biochemically distinguishing phenotypes of Gaucher's disease. This enables one to identify neurologic and non-neurologic phenotypes presymptomatically. A positive correlation has been found between the neuropathologic changes in Gaucher brain with the content of glucocerebroside. Finally, a clinical trial of enzyme replacement is being conducted in Gaucher's disease. The application of gene transfer to human disease is under consideration.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01NS02665-01 DMN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Mapping Functional Domains of Lysosomal Enzymes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
Edward I. Ginns, M.D., Ph.D., Mol. & Med. Genetics Section, DMNB, NINCDS
Others: John A. Barranger, M.D., Ph.D., Assoc. Chief, DMNB, NINCDS; Prabhakara Choudary, Ph.D., Barbara Stubblefield, Suzanne Winfield, Shoji Tsuji, M.D., Ph.D., June Mayor, Mary LaMarca, and Brian Martin, Ph.D., and Carl Lauter, MMGS, DMNB, NINCDS; Gary Murray, Ph.D., CITS, DMNB, NINCDS;

COOPERATING UNITS (if any)

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Molecular & Medical Genetics Sect./Clinical Investigations & Therapeutics Sect.

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.9	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
☒ (b) Human tissues
☐ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The accessibility of lysosomal enzymes to their substrates is affected by their subcellular compartmentalization. Routing of enzymes through the cell has been shown to be influenced by mutations occurring in the lysosomal enzyme as well as in other proteins. Due to the lack of sufficient quantities of homogeneous normal and mutant enzymes for biochemical and structural studies, we felt that it was necessary to isolate the cDNA encoding specific enzymes and by in vitro mutagenesis recreate the consequences of mutations on enzyme activity and structure. To accomplish this end, initially using Gaucher's disease as a model, we isolated and sequenced the cDNA encoding all of human glucocerebrosidase. We have described the leader sequence for this enzyme and thus have identified that portion of glucocerebrosidase that effects translocation of the enzyme to the cisternae of the endoplasmic reticulum. In order to map the other domains responsible for oligosaccharide addition and processing, substate hydrolysis, lysosomal routing and membrane association, we have synthesized oligonucleotides that are being used to mutagenize the cDNA for glucocerebrosidase. Using retroviral constructs, the cDNA was transferred to mammalian host cell lines to reconstruct Gaucher variants. This provides an in vitro cell culture model in which the compartmentalization and function of the transferred protein can be directly correlated with specific changes in the cDNA and hence protein domains. This research will provide a more rational basis for the development of therapeutic strategies using gene or gene product replacement.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	**PROJECT NUMBER** Z01NS02666-01 DMN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Application of Gene Transfer to the Correction of Inherited Enzyme Deficiencies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Edward I. Ginns, M.D., Ph.D., Molecular & Medical Genetics Section, DMNB, NINCDS
Others: John A. Barranger, M.D., Ph.D., Assoc. Chief, DMNB, NINCDS;
Prabhakara Choudary, Ph.D., Barbara Stubblefield, Suzanne Winfield,
Nancy Miller, Ph.D., Shoji Tsuji, M.D., Ph.D., June Mayor, Mary LaMarca,
and Brian Martin, Ph.D., & Carl Lauter, MMGS, DMNB, NINCDS;
Gary Murray, Ph.D., CITS, DMNB, NINCDS;
Dr. Richard Mulligan and Dr. Connie Cepko, Whitehead Institute, MIT.

COOPERATING UNITS *(if any)*
Massachusetts Institute of Technology, Whitehead Institute, Cambridge, MA

LAB/BRANCH
Developmental and Metaboalic Neurology Branch

SECTION
Molecular & Medical Genetics Sect./Clinical Investigations & Therapeutics Sect.

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.3	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The isolation of cDNA containing the full sequence encoding human glucocerebrosidase has permitted the use of this enzyme in model studies to correct inherited enzyme deficiencies using recombinant methodologies, specifically gene transfer. Particularly suited for gene therapy are those disorders (such as Gaucher's disease) where the storage of undegraded substrate is confined to cells having an accessible precursor population. In these cases, the transfer of normal genes to stem cells in bone marrow would be both rational and desirable. Using information derived from protein studies, the products of gene transfer in mutant cells can be compared to that of normal cells, and the likelihood for success of a particular construct and gene integration rationally predicted. Although we have been successful in utilizing retroviral vectors to transfer and express glucocerebrosidase in host mouse and human cell lines, prerequisites for human gene therapy experiments include sustained expression of the transferred gene during subsequent cell generations and the absence of recombination events detrimental to the host. These aspects are being defined. To better characterize the gene transfer and expression mechanisms Type 2 Gaucher cell lines were utilized as recipients of the retroviral constructs. In this model, monoclonal antibody 8E4 does not recognize the Type 2 variant glucocerebrosidase. Thus, these cells provide a host cell line lacking the normal enzyme epitope recognized by 8E4, and they allow the monitoring of the degree of restoration of both enzyme activity and protein epitopes resulting from gene transfer. Following the demonstration of restored glucocerebrosidase levels to these as well as Type 1 and Type 3 cell lines in culture, the transfer of the glucocerebrosidase gene to bone marrow stem cells will be evaluated using mice and non-human primates. The goal of this research is the application of these recombinant DNA therapeutic strategies to Gaucher's disease and other genetic disorders.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02681-01 DMN

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetic Studies of the Mucopolysaccharidoses IH, IH/S and IS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Edward I. Ginns, M.D., Ph.D., Molecular & Medical Genetics Sec., DMNB, NINCDS
Others: John A. Barranger, M.D., Ph.D., Assoc. Chief, DMNB, NINCDS;
Prabhakara Choudary, Ph.D., Brian Martin, Ph.D., Barbara Stubblefield, Suzanne Winfield, Nancy Miller, Ph.D., Shoji Tsuji, M.D., Ph.D., June Mayor, Mary LaMarca, and Carl Lauter, MMGS, DMNB, NINCDS;
Gary Murray, Ph.D., CITS, DMNB, NINCDS.

COOPERATING UNITS (if any)

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Molecular & Medical Genetics Sect./Clinical Investigations & Therapeutics Sect.

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.9	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Phenotypic heterogeneity seen within specific genetic disorders provides an opportunity to examine the mutational events responsible for the observed clinical diversity. Patients with mucopolysaccharidoses IH, IH/S or IS all have a deficiency of α-L-iduronidase, but the abnormality of enzymatic activity alone is insufficient to explain the wide differences in severity of symptoms in the three major variants of the disorder. As has been demonstrated for other lysosomal disorders, it is likely that a description of the effect of mutations on the biosynthesis, intracellular compartmentalization and degradation of α-L-iduronidase will shed light on the significance of transcriptional, translational or other protein processing abnormalities that result in the neurologic and non-neurologic features of these diseases. The aim of these studies is to define the abnormal biochemistry of this group of mucopolysaccharidoses and to characterize the factors responsible for variations in symptoms among patients in molecular terms. To achieve this goal, the α-L-iduronidase was purified from human placenta. This has allowed the study of both the biochemical and immunological properties of this enzyme. Pulse-chase and western analyses of α-iduronidase using normal and mutant cell lines were performed to elucidate protein polymorphisms that may prove to be characteristic of the individual phenotypes. Studies dealing with the isolation of the gene are in progress. The isolation of cDNA clones encoding normal human α-L-iduronidase permit a more detailed study of the chromosomal locus, gene variants, as well as the factors controlling expression of the gene. These studies allow formulation of therapeutic strategies utilizing both gene product and recombinant DNA approaches.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01NS02682-01 DMN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Molecular Genetics of Lysosomal Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
Edward I. Ginns, M.D., Ph.D., Molecular and Medical Genetics Sec., DMNB, NINCDS
Others: John A. Barranger, M.D., Ph.D., Assoc. Chief, DMNB, NINCDS
Prabhakara Choudary, Ph.D., Brian Martin, Ph.D., Barbara Stubblefield, Suzanne Winfield, Nancy Miller, Ph.D., Shoji Tsuji, M.D., Ph.D., June Mayor, Mary LaMarca, and Carl Lauter, MMGS, DMNB, NINCDS;
Gary Murray, Ph.D., CITS, DMNB, NINCDS.
Drs. J. Tager & A. Schram, Dept. of Biochem., Univ. of Amsterdam

COOPERATING UNITS (if any)
Department of Biochemistry, University of Amsterdam, The Netherlands

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Molecular & Medical Genetics Sect./Clinical Investigations & Therapeutics Sect.

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.3	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We approached the characterization of the mutations resulting in the inherited lysosomal disorders by addressing the genetic and molecular variants in the synthesis and intracellular routing of these enzymes. Using Gaucher's disease as a model, we demonstrated specific protein polymorphisms for each observed phenotype. We further demonstrated that phenotypic heterogeneity seen within these inherited disorders may be a consequence of different mutations, each affecting enzyme activity and influencing the processing, compartmentalization and/or stability of the lysosomal hydrolases. Although the understanding of the specific mechanisms responsible for clinical diversity has increased as a consequence of protein analyses, many of the primary pathophysiological processes have still not been well described. Recently we extended our understanding of these disorders by the application of recombinant DNA techniques to elucidate the structure of the gene(s) for these enzymes. The cDNA clones that we isolated have permitted the more complete description of the transcriptional and translation events. These cDNA clones have also permitted the localization of the gene for this enzyme by in situ hybridization, as well as the isolation of genomic clones. Restriction fragment length polymorphisms (RFLP's) have been identified. Northern and S1 nuclease analyses provide further details of the structure of the normal and mutant genes. The molecular mechanisms leading to nervous system involvement within these disorders have also been investigated. Western analysis and pulse-labelling of normal and mutant glucocerebrosidase demonstrate that the several phenotypes of Gaucher's disease are caused by different mutations. We have also constructed human brain libraries from which cDNA for the lysosomal enzymes has been isolated. A comparison of these genes to those from non-neural tissues should provide further information on the regulation of tissue specific expression. The results of this research will provide a more rational foundation for therapeutic endeavors for these inherited disorders.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02683-01 DMN

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of Eukaryotic Shuttle Vectors for Human Gene Transfer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Prabhakara V. Choudary, Ph.D., Senior Staff Fellow, DMNB, NINCDS
Others: John A. Barranger, M.D., Ph.D., Assoc. Chief, DMNB, NINCDS;
Edward Ginns, M.D., Ph.D., Brian Martin, Ph.D., Barbara Stubblefield, Suzanne Winfield, Nancy Miller, Ph.D., Shoji Tsuji, M.D., Ph.D., June Mayor, Mary LaMarca, and Carl Lauter, MMGS, DMNB, NINCDS;
Gary Murray, Ph.D., CITS, DMNB, NINCDS;
Dr. Rchard Mulligan and Dr. Connie Cepko, Whitehead Institute, MIT.

COOPERATING UNITS *(if any)*

Massachusetts Institute of Technology, Whitehead Institute, Cambridge, MA;
Meloy Laboratories, Springfield, VA

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Molecular & Medical Genetics Sect./Clinical Investigations & Therapeutics Sect.

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.8	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In mammalian cells, gene transfer is accomplished traditionally by DNA-mediated transfection, a general procedure for introduction of foreign DNA either singly or in combination with a dominant selectable marker. Taking advantage of this, we are studying the transfer of cDNA for human glucocerebrosidase to mouse cells via bovine papilloma virus (BPV) vector and the synthesis, in culture, of large amounts of the enzyme in biologically active form. Using this recombinant product, we are addressing the molecular and cell biological issues related to biosynthesis, compartmentalization, processing and function of lysosomal glucocerebrosidase. These studies complement work on perfecting enzyme replacement techniques.

In addition, a major goal of our laboratory is to develop novel therapeutic strategies for inborn errors of metabolism. Of the approaches that have been designed in recent years for this purpose, the most promising one appears to be to infect pluripotent stem cells with chimeric retroviruses carrying a correct copy of the defective gene. Using retroviral shuttle vectors of the pZip neo family in conjunction with the specialized host cell lines, Viz., $\psi 2$ and ψAM, that produce helper-free recombinant retroviruses, we have generated high-titer stocks of transmissible chimeric retroviruses carrying a cDNA copy of the human glucocerebrosidase gene. The mouse cell lines as well as the human fibroblasts infected with these replication-defective virus stocks revealed appropriately integrated copies of the proviral DNA and produced antigenically active human glucocerebrosidase protein. We are currently focussing on the manipulation of the insert DNA adduct in the retroviral constructs to obtain enzymatically active protein. The results obtained in this project clearly demonstrate the feasibility of retroviral vector system for human gene transfer and somatic cell gene therapy.

TAB 15 -- EXPERIMENTAL THERAPEUTICS BRANGH -- (ETB)

ANNUAL REPORT

October 1, 1984 through September 30, 1985

Experimental Therapeutics Branch

National Institute of Neurological and
Communicative Disorders and Stroke

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ANNUAL REPORT

October 1, 1984 through September 30, 1985

Experimental Therapeutics Branch, IRP
National Institute of Neurological and Communicative Disorders and Stroke
Thomas N. Chase, M.D., Chief

The Experimental Therapeutics Branch directs its investigative efforts towards the rational development of improved pharmacotherapies for disorders of the human central nervous system. An integrated program of fundamental and applied research seeks to define relationships between clinical signs of brain dysfunction and specific alterations in neuronal transmission; based on a detailed understanding of synaptic mechanisms and of potential sites for pharmacologic intervention, novel therapeutic approaches are developed to modify the affected system and thus improve clinical function. Branch research, at both clinical and preclinical levels, remains focused on the dopamine system and closely interactive neuronal pathways in relation to extrapyramidal and dementing disorders.

The Branch is currently organized into four highly integrated components: Dr. John Kebabian's Biochemical Neuropharmacology Section carries out basic biochemical and pharmacologic studies of dopamine receptor mechanisms. Dr. Judith Walters' Physiological Neuropharmacology Section evaluates interactions between the dopamine system and other transmitter pathways within the basal ganglia. Dr. Thomas O'Donohue's Neuroendocrinology Unit investigates peptidergic systems involved in cognitive and motor function. Dr. Thomas Chase's Pharmacology Section explores transmitter abnormalities and pharmacologic interventions in dementing and extrapyramidal disorders.

BIOCHEMICAL NEUROPHARMACOLOGY SECTION

During FY 85, the Section continued to focus upon its two areas of traditional strength, dopamine receptor pharmacology and pituitary cell biology. During this FY, the Section Chief, Dr. Kebabian, received a Special Recognition Award from the PHS for his formulation of the 'two dopamine receptor' hypothesis.

The pharmacological investigations of the Section during the current FY involved the development of an iodinated ligand for the D-1 dopamine receptor. The molecule (developed in collaboration with Carl Kaiser of SK&F Laboratories) is an analogue of SCH 23390, a selective D-1 dopamine receptor antagonist. The ligand is formed by replacing the chlorine at position 7 in SCH 23390 with (^{125}I). The iodinated molecule retains the ability to discriminate between the D-1 and D-2 dopamine receptors; its high specific activity and high affinity towards the D-1 receptor permits binding studies to be performed on relatively small quantities of striatal tissue. Having synthesized the analogue, the Section demonstrated that the molecule interacted with a binding site resembling the D-1 dopamine receptor. The ligand interacted with two categories of binding site differing in affinity for the ligand (0.1 nM and 2.7 nM). Both of these sites resemble the D-1 dopamine receptor. The iodinated ligand competes in a stereoselective manner with both agonists (e.g. SKF 38393) and antagonists (e.g. SKF 83566 or SCH

23390) of the D-1 receptor. The binding site for the ligand does not recognize (or recognizes with low affinity) drugs selective for the D-2 receptor.

The (^{125}I)-ligand has proven to be a useful tool for characterization of the D-1 receptor in the striatum. In binding studies, it is possible to directly determine the affinity of drugs for the binding site. The Section has utilized this aspect of the binding assay to characterize the affinity of various aporphines for the D-1 receptor. The specific question being asked was the importance of free hydroxyl groups versus methoxy groups at positions 10 and 11 in the aporphine nucleus for binding of the 6aR or 6aS enantiomers of aporphines to the D-1 receptor.

The Section has continued its studies of the 'desensitization' of the D-2 receptor induced by exposure to agonists. Previously, the Section had reported that exposure to agonists diminished the ability of D-2 agonists to inhibit adenylate cyclase activity. During the current FY, the Section used the newly developed iodinated D-2 receptor selective ligand NAPS to characterize the effects of agonist exposure upon the properties of the NAPS binding sites in the intermediate lobe. Exposure of agonists diminished the affinity of NAPS towards the specific binding sites. The total number of sites was not decreased. Exposure to agonist diminished the affinity of the specific binding site for agonists approximately 8-fold; the effect upon antagonist affinity was less striking.

During FY 85, the Section continued to study the involvement of cAMP in the process of pituitary hormone release. The section showed that in the 7315c tumor cell (which secretes prolactin) forskolin, which elevates the production of cAMP, increases the amount of hormone released from the cells in response to a challenge with potassium or ionomycin without affecting the rise in calcium produced by either agent. Consequently, cells exposed to forskolin release more hormone in response to a fixed rise in the concentration of cytosolic calcium.

The Section has also begun to develop a preparation of permeabilized pituitary cells. These permeabilized cells secrete hormone in response to calcium (in the presence of Mg and ATP). It is anticipated that these cells will be of use in studying the process of role of cAMP in the process of hormone release.

NEUROENDOCRINOLOGY UNIT

Pharmacology and Cellular Biology of Peptidergic Neurons

The most recently identified and the major known class of neurotransmitters and hormones is comprised of peptides. The goal of the Unit is to develop an understanding of the basic regulatory mechanisms in cells which secrete peptides, and through this understanding, develop novel pharmacotherapeutic approaches and agents for manipulating peptidergic systems. Two projects are ongoing. The first studies the regulation of biosynthesis of peptides. The primary model under investigation is the opiomelanocortin containing neuronal and endocrine system which secretes ACTH, α-MSH and β-endorphin. These peptides are derived from a single prohormone (pro-opiomelanocortin or POMC) and influence arousal and cognitive processes through interactions with central MSH receptors and analgesia through interactions with mu and delta opioid receptors. The second investigation is focused on studies of an endogenous peptide ligand which interacts with the sigma opioid receptor.

1. Regulation of biosynthesis of peptides

Biosynthesis of peptides occurs in three steps. First, the process is initiated by signal transduction between cell surface receptors and the biosynthetic mechanisms. Second, peptide prohormone and processing enzymes are synthesized. Third, the final secretory form of the peptide is generated by cleavage and modification of the prohormone by post-translational processing enzymes.

In the last three years, the Unit investigated the third biosynthetic step -- the roles and regulation of post-translational processing of POMC. It was found that post-translational processing of the POMC-derived peptides dramatically changes their biological and behavioral activity. The POMC derived peptides, α-MSH and β-endorphin, bind to different postsynaptic receptors but have extensive interactions. In FY 84, it was found that the extent of post-translational processing of POMC was not only tissue specific but also varied in different physiological situations. For example, it was observed that there are different ratios of POMC derived peptides in different tissues and this ratio changes in physiological situations. In FY 85, the Unit has continued investigations of regulation of post-translational processing of POMC and post-translational processing enzymes. It was found that the biosynthesis of POMC and certain POMC post-translational processing enzymes is coordinately regulated and can be co-induced or co-inhibited by regulation of cell surface receptors. Similar to the case for POMC, pro-tachykinin is the prohormone for two major neuropeptides -- substance P and substance K which were found to interact with different postsynaptic receptors in FY 85. Also like POMC derived peptides, the substance P to substance K ratios were inconsistent in different tissues. Unlike POMC, however, the different ratios appear to be due to different post-transcriptional processing of pro-tachykinin mRNA and not selective post-translational processing of pro-tachykinin.

In the last two years, the Unit investigated the second biosynthetic step -- the mechanism of induction of POMC biosynthesis primarily using the POMC-containing cells of the intermediate lobe of the pituitary as a model. It was found that there are temporal differences in the way a neuroendocrine system regulates peptide biosynthesis. After long-term stimulation or inhibition, the secretory cells of the intermediate lobe proliferate or die in response to the requirement for POMC secretion. After a moderate length of stimulation or inhibition, inactive cells are recruited or active cells turned off. After acute stimulation, the biosynthesis of POMC is induced by increasing the intracellular content of POMC mRNA. The increase in POMC mRNA could be due to either a decrease in degradation rate of POMC or an increase in the POMC mRNA transcription rate. In cultured corticotroph tumor cells, we have found that stimulation of corticotropin releasing factor (CRF) receptors on the cell surface induces transcription of POMC mRNA within minutes. It therefore appears that the primary site of POMC biosynthesis regulation is at the transcriptional level.

In FY 85, the Unit began studies on the mechanism of signal transduction between cell surface receptors and the biosynthetic process. The induction of POMC gene transcription was found to be a cAMP-protein kinase A dependent mechanism in both corticotrophs and melanotrophs. It was also found that the diacylglycerol-protein kinase C system is involved in regulating biosynthesis in corticotrophs. A systematic study of protein kinase A and protein kinase C substrates for phosphorylation was begun to determine the putative third messengers involved in transmitting information from cell surface receptors to the nucleus.

2. An endogenous peptide ligand for the sigma opioid receptor

β-endorphin, enkephalin and dynorphin have been identified as endogenous peptide ligands for the mu, delta and kappa opioid receptors. These receptors appear to be involved in the analgesic and reward processes of opioids. The sigma opioid receptor, according to the original classification, mediates the psychotomimetic properties of certain opiates. In FY 83, the Unit identified a peptide in the central nervous system which binds to the sigma opioid or phencyclidine (PCP) receptor. In addition, this peptide shares behavioral and electrophysiological activities of PCP. The peptide has been purified to homogeneity and was found in highest concentrations in cerebral cortex and hippocampus.

Ligands used to identify the sigma opioid receptor include phencyclidine, SKF 10,047 and dexoxadrol. All of these compounds produce psychotomimetic actions in rats and humans and were thought to do so by interactions with a single receptors. In FY 85, we found that the sigma opioid receptor is not a single site but is composed of at least two, and probably three different binding sites. The highest density of all the subtypes of receptors is located in the cerebral cortex and hippocampus. These sites which contain the highest densities of the PCP-like peptide. The localization of receptors and peptide in these sites is consistent with both the psychotomimetic and cognitive effects of these compounds.

In FY 84, the Unit developed Metaphit, the first compound that can be used as a PCP/sigma opioid antagonist. Metaphit is a PCP receptor acylating agent. In FY 85, the mechanism of action of Metaphit was explored. The compound was found to have some agonist actions when it initially binds to the receptor but subsequently specifically blocks the long-term behavioral and electrophysiological effects of phencyclidine. It therefore appears that the agonist effects of Metaphit and, perhaps, PCP are due to the receptor binding event and not due to receptor occupancy. Metaphit or a derivitive of this compound may find clinical utility for the treatment of PCP overdose.

PHYSIOLOGICAL NEUROPHARMACOLOGY SECTION

The Physiological Neuropharmacology Section has continued to investigate the role of specific neurotransmitters in regulating neuronal activity in extrapyramidal systems. In the past year, research has focused on defining the nature and function of the dopamine receptors associated with the nigrostriatal dopamine neurons in order to elucidate the role of this neuronal system in regulating basal ganglia output. We have also carried out studies investigating the involvement of the substantia nigra in epileptic seizure propagation and the potential for excitatory amino acids to affect substantia nigra neuronal activity. Our goal is to better understand how specific neurotransmitter systems affect information processing in the basal ganglia and to develop improved pharmacological treatment for neurological disorders involving the basal ganglia and substantia nigra regions.

1) Structure and Function of Dopamine Receptors in the Basal Ganglia and Substantia Nigra

Dopamine receptors in the CNS and in the periphery have been classified as either D-1 receptors, coupled with adenylate cyclase; or D-2 receptors, independent of, or negatively coupled to adenylate cyclase. Recently, much interest has focused on the question of whether the dopamine receptors located on the dopamine neurons, termed dopamine autoreceptors, are, in fact, identical to the postsynaptic D-2 dopamine receptors, or whether the autoreceptors may constitute a distinct subset of receptors which could be selectively stimulated by a highly specific agonist. Such an agonist might have some therapeutic advantages in the treatment of schizophrenia and tardive dyskinesia. To investigate the possibility that the dopamine autoreceptors and the postsynaptic "D-2" receptors may be differentiated pharmacologically, we have determined the relative potencies of a series of dopamine agonists on the autoreceptors and are in the process of obtaining a similar potency series for the postsynaptic receptors.

For the dopamine autoreceptors, we have found the relative potencies of a series of systemically administered dopamine agonists to be: apomorphine = LY 171555 = RU 24926 = EMD 36 362 = pergolide = lisuride (+)-3-PPP = lergotrile (-)-3-PPP LSD SKF 38393. This potency series is very similar to that described by Goldberg and associates for the DA-2 receptor on canine renal arteries. Iontophoretic studies are producing similar results. The results support the idea that the dopamine autoreceptors are of the classic D-2 subtype.

To investigate the nature and function of the postsynaptic dopamine receptors in the basal ganglia and substantia nigra, we have been studying the direct and indirect effects of dopamine drugs on the activity of neurons in the globus pallidus and substantia nigra pars reticulata. These two areas, not normally thought of as being innervated by dopamine cells, appear to have dopamine receptors and may, in fact, be exposed to dopamine released from the substantia nigra dopamine neurons. The globus pallidus has a sparse but widespread dopamine innervation from the substantia nigra. While dopamine dendrites are believed to release dopamine in the vicinity of the neurons in the pars reticulata of the substantia nigra. Thus, the globus pallidus and substantia nigra pars reticulata neurons can also be affected both directly and indirectly by systemically administered dopamine agonists and antagonists. The neurophysiological actions of systemically administered dopaminergic agents on cells in the substantia nigra pars reticulata and the globus pallidus reflect the net effects of these drugs on the postsynaptic dopamine receptors.

Previous studies have revealed that pallidal neurons are consistently stimulated by systemically administered nonselective dopamine agonists such as apomorphine which interact with both D-1 and D-2 type dopamine receptors. The selective D-1 agonist, SKF 38393, does not mimic the effects of apomorphine on these neurons, suggesting that the D-1 receptors are not involved with mediating this response. Moreover, when we examined the relative effects of 4 agonists, apomorphine, pergolide, LY 171555 and RU 24926, all equipotent on the dopamine autoreceptors, we found that the putatively selective D-2 agonists, LY 171555 and RU 24926, were significantly less efficacious than the two nonselective agonists. Thus, at least some of the postsynaptic receptors mediating the haloperidol-reversible effects of apomorphine and pergolide on pallidal activity appear to be relatively insensitive not only to the D-1 agonist, but to the selective D-2 dopamine agonists as well. The greater effects of the non-selective agonists on pallidal activity do not appear due to their stimulation of both receptor subtypes. Moreover, LY 171555 is not acting as a partial agonist at postsynaptic sites. These results raise the interesting possibility that some postsynaptic dopamine receptors mediating the increases in pallidal activity are not classical D-1 or D-2 type receptors.

The relative effects of systemically administered selective D-1 and D-2 agonists and antagonists on firing rates in the substantia nigra pars reticulata, results have been consistent with those observed in the globus pallidus. The D-1 agonist SKF 38393 does not produce in normal rats the magnitude of variability of substantia nigra pars reticulata response induced by apomorphine. However, neither does the D-2 agonist, LY 171555 even though, as described above, this drug and apomorphine are equipotent at inducing changes in dopamine cell activity mediated through dopamine autoreceptors. Similar results are seen in rats with supersensitive dopamine receptors following lesion of the dopamine neurons with 6-hydroxydopamine. Thus, in substantia nigra pars reticulata as well as in the globus pallidus, a selective D-2 agonist which is equipotent with apomorphine at inhibiting the substantia nigra pars compacta dopamine cells does not appear to be exerting effects as great as those of apomorphine.

In our investigations of the effects of drugs selective for specific subsets of dopamine receptors we have also found evidence for a modulatory interaction between D-1 and D-2 receptors when we block D-1 receptor. In both of the globus pallidus and substantia nigra pars compacta, D-1 receptor blockade attenuates the effects of the dopamine agonists on neuronal activity, indicating that the two receptor subtypes may interact in some way to influence basal ganglia output. The effects of the dopamine agonists and antagonists on pallidal activity correlate quite well with their effects on rat behavior, suggesting that understanding how and why dopamine and dopaminergic drugs act to affect globus pallidus activity may provide some insight into the mechanisms by which these substances and the receptors which mediate their actions affect behavior.

2) Role of the Substantia Nigra Pars Reticulata in Epilepsy

Recent reports have suggested that enhancement of GABAergic transmission within the substantia nigra prevents the motor manifestations of both chemically-induced and kindled seizures. To explore the possibility that the substantia nigra directly transmits seizure activity from rostral sites of origin to target structures; and/or 2) simply produces a tonic seizure facilitating action of other structures which themselves directly transmit the seizure activity, we recorded single unit activity and EEG of substantia nigra neurons during electrical seizures induced by stimulation of amygdala in kindled rats. The most striking finding was that substantia nigra neurons in the kindled animals exhibited a dramatic change in firing pattern during the electrical seizure which consisted of bursts temporally correlated to the specific components of the EEG. The dramatic change in firing pattern, time-locked to specific components of the EEG indicates that the substantia nigra is directly transmitting seizure activity in the kindled rat.

To gain further insight into the possible importance of the substantia nigra as a therapeutic target in the treatment of epilepsy, we have evaluated the effects of a diverse group of anticonvulsant drugs on the firing rates of the substantia nigra pars reticulata neurons, and attempted to correlate these effects with the drugs' anticonvulsant profiles and/or presumed mechanisms of action. Phenytoin and carbamazepine did not alter neuronal firing rates at any dose. Conversely, both diazepam and clonazepam partially inhibited firing, although clonazepam was approximately 16 times more potent in eliciting equivalent degrees of inhibition. Phenobarbital and valproic acid also partially inhibited cell firing but inhibition occurred only at the highest doses administered. Unlike the above drugs, ethosuximide markedly increased firing. These results suggest a non-uniform profile of drug action of tonic activity of pars reticulata neurons with consistent inhibitory effects produced only by drugs with known or proposed GABAergic mechanisms. Whether the anticonvulsant agents might have different profiles of action in suppressing the phasic bursting activity associated with seizure propagation in this brain region, as described above, remains to be determined.

3) Role of Glutamate and Related Neurotransmitters in the Substantia Nigra

In order to assess the effects of glutamatergic input on substantia nigra activity, we have iontophoretically applied excitatory amino acid agonists and antagonists in substantia nigra and examined their effects on substantia nigra neuronal firing. The actions of the different agonists and antagonists examined suggested that both the dopamine and the pars reticulata neurons have 2 and perhaps 3 different excitatory amino acid receptors. Preliminary results suggest an N-methyl-D-aspartate (NMA) prefering receptor may be tonically stimulated on some cells in the pars reticulata. Moreover, the regular and striking increases in rate sustained by the dopamine cells during NMA application represents a previously undescribed firing mode and indicates that these cells can be activated in more than one way; in addition to their burst firing response to stimulation by glutamate and other agents, these studies show they also have a mechanism for firing more rapidly without becoming depolarized.

PHARMACOLOGY SECTION

The Section conducts clinical and laboratory studies linking the Branch's basic research efforts with the neurologic patient. Clinical investigations seek to associate the status of a particular transmitter system with specific signs of extrapyramidal or cognitive dysfunction. Evidence bearing on such relationships provides the basis for preclinical studies of pathophysiological mechanisms and novel pharmacotherapeutic interventions, especially those involving the dopamine system and interacting peptidergic pathways. Pathophysiologic hypothesis and drug therapies deriving from these laboraory studies are then submitted to clinical evaluation.

Dementing Disorders

1. Cerebral Imaging Studies.

Clinical investigations of Alzheimer's disease during the past year have extended the characterization of the regional pattern of cortical dysfunction previously discovered by the Branch. A comparison of results from positron emission tomography (PET) scans following fluorodeoxyglucose (FDG) in Alzheimer patients with age-matched controls indicates that while most of the cerebral cortex is abnormal (the most notable exception being the primary motor-sensory areas) involvement is greatest in the parietal association cortex, where the metabolic reduction is twice that found in represenative areas of frontal or anterior temporal lobe. This cortical distribution is consistent with the findings of several published neuropathologic studies and has now been corroborated by results from other PET centers.

2. Neuropsychological studies.

Preponderant involvement of the parietal association cortex, a site of cortical integration of visual, auditory and somatosensory inputs, is consistent with our finding that the major clinical features of Alzheimer's disease include aphasias, apraxias, and agnosias. Current investigations seek a more precise understanding of the pathophysiology of these abnormalities. A recently completed analysis of the cortical representation of dyspraxia in right handed Alzheimer patients failed to reveal any correlation between the response to motor tasks given by spoken command or visual demonstration and the degree of overall dementia. Performance to command, however, was most closely associated with scores on neuropsychological tests dependent on verbal proficiency and correlated most strongly with glucose utilization in the left inferior frontal and superior temporal areas. In contrast, the ability to imitate related most closely with performance on tests of visual-spatial skill and correlated best with cortical function in portions of the right posterior parietal lobe.

3. GABA System Studies.

Recent results suggest that spinal fluid levels of gamma aminobutyric acid (GABA) are consistently reduced in Alzheimer patients. Correlations between spinal fluid GABA concentrations and regional glucose utilization rates suggest that GABA neuron abnormalities occur predominently in the frontal lobe in contrast with the mainly parietal localization of the cortical metabolic dysfunction in Alzheimer's disease. In a further effort to evaluate the contribution of GABA system degeneration to Alzheimer dementia, we have recently completed a clinical trial of THIP, which acts relatively specifically to stimulate $GABA_A$ receptors. Patients selected for study had spinal fluid GABA levels substantially below those of normal controls or otherwise unselected Alzheimer patients. No consistent effect on cognitive function was found, even at doses where THIP appeared to interact with central GABA receptors. GABA system dysfunction in Alzheimer's disease may thus be a secondary rather than primary deficit.

4. Cholinergic System Studies.

The evaluations of post mortem tissues from individuals with histologically proven Alzheimer's disease now suggests that decreases in the activity of the acetylcholine synthesizing enzyme, choline acetyltransferase, are rather uniformly distributed throughout the cerebral cortex; reductions in posterior parietal areas identified by PET as being most severely abnormal in Alzheimer's disease are not significantly different from those in the less severely involved frontal regions. It is thus unlikely that the degeneration of cholinergic projections to the cerebral cortex explain the pattern of FDG reductions found in Alzheimer's disease. The lack of therapeutic efficacy of drugs believed to potentiate cholinergic transmission by increasing precursor availability or blocking transmitter degradation might reflect either the relative scarcity of residual presynaptic cholinergic terminals or the possibility that these cholinergic terminals synapse with cortical neurons which are also affected by the degenerative process. In an attempt to distinguish these possibilities, we are now completing a clinical trial of RS 86, a selective yet potent agonist at postsynaptic M-1 and M-2 muscarinic receptors. The available results do not encourage a very optomistic view of the clinical efficacy of this drug.

5. Somatostatin System Studies

Spinal fluid somatostatin levels are substantially reduced in Alzheimer's disease. The magnitude of this decrement correlates most closely with glucose utilization in precisely those posterior parietal areas which appear most abnormal in PET-FDG studies. Available data further suggest a close relation between the degree of somatostatin reduction in lumbar spinal fluid and the severity of primary clinical features of Alzheimer's disease. Studies of somatostatin in post mortem specimens indicate that while reductions in excess of 50 percent occur in the posterior parietal area when compared with age-matched controls, concentrations of this neuropeptide in the frontal cortex are essentially unchanged, a pattern consistent with PET-FDG findings. Taken together our results suggest that cortical somatostatin neurons could play an important role in the pathophysiology of Alzheimer dementia. Since cortical neuropeptide Y levels remain normal in this disorder, we conclude that neurons containing both neuropeptide Y and somatostatin are spared, while those which contain somatostatin either alone or in combination with some other, as yet unidentified, neurotransmitter are particularly affected.

7. Vasopressin System Studies.

We have previously reported that the systemic administration of lysine vasopressin failed to alter cognitive function in patients with Alzheimer's disease. Nevertheless, in the experimental animal peripherally administered vasopressin or its analogs have been reported to exert behavioral effects which appear to link this neuropeptide to memory consolidation and retrieval mechanisms. These conclusions are largely based on experiments which involve aversive conditioning techniques. Since the effects of vasopressin on appetitively-motivated behaviors might have an even greater relevence to its ability to influence human cognition, we have recently evaluated both arginine and des-glycinamide arginine vasopressin in relation to steady state operant responding. Neither peptide significantly altered fixed-interval response patterning. The results thus failed to support the hypothesis that vasopressin improves memory or that it reduces the value of reinforcing stimuli. Indeed, our findings suggest that the behavioral effects of vasopressin should be viewed in light of its aversive peripheral actions.

Extrapyramidal Disorders

1. On-off Phenomena in Parkinson's disease.

After several years of levodopa treatment most parkinsonian patients become increasingly disabled by short term fluctuations in motor performance. We have previously reported a close relation between the variations in plasma dopa levels attending oral levodopa treatment and the fluctuations in antiparkinsonian response. Recent studies suggest that progressive reductions in the half-life of this compound in the general circulation cannot explain these motor variations, since no differences in peripheral clearance mechanisms for levodopa could be found between patients with wearing-off or on-off phenomena and those who are stable responders. It does appear, however, that central pharmacokinetic or pharmacodynamic factors, which initially serve to stabilize the antiparkinsonian response to varying circulating levodopa levels, are altered in patients who evidence motor fluctuations: The duration of the antiparkinsonian action of levodopa appears significantly shorter in patients manifesting wearing-off and especially in those with on-off phenomena than in stable responders.

Current investigators also seek to determine whether efforts to attenuate the variations of circulating levodopa levels associated with the oral administration of this drug might confer significant therapeutic benefit. We have previously reported the ability of short term levodopa infusions to stabilize the antiparkinsonian response in patients with wearing-off effects. We have now begun an evaluation of prolonged levodopa infusions in patients with either wearing-off or on-off responses, using portable pumps under fully ambulatory conditions inside the hospital and following discharge. Patients in both levodopa response categories appear to be achieving relatively stable plasma drug levels as well as less frequent and lower amplitute motor fluctuations.

The foregoing results have prompted reconsideration of pharmaceutical approaches to the maintenance of stable plasma levodopa levels. Our previous trials of sustained release levodopa formulations met with little success. Several new controlled release preparations containing levodopa plus carbidopa have now been tested in a small group of parkinsonian patients for periods of up to 9 weeks. Plasma dopa levels were substantially more constant than with orally administered levodopa. Clinically, some patients evidenced an unmistakable attenuation in motor fluctuations compared with those occurring with standard levodopa preparations. Additional benefits of these sustained release preparations included the convenience of less frequent dosing, improved nocturnal sleeping, and less early morning akinesia or dystonia.

2. Noradrenergic mechanisms in Parkinson's disease.

Considerable evidence suggests that noradrenergic mechanisms participate in the regulation of human extrapyramidal function. We have previously reported that procedures which stimulate the release of norepinephrine, diminish the antiparkinsonian response to a stable dose of intravenously infused levodopa. More recently, the ability of a mixed (beta 1 plus beta 2) beta-adrenoceptor antagonist, nadolol, which is largely excluded from the central nervous system was tested for its ability to diminish tremor in parkinsonian patients. When nadolol was added to the patients' regular therapeutic regimen, resting as well as action tremor were substantially reduced. No centrally mediated side effects occurred.

3. Preclinical Cholecystokinin Studies.

Recent evidence suggests that cholecystokinin octapeptide (CCK-8) containing neural systems may contribute to the pathophysiology of certain neuropsychiatric disorders, especially those which involve dopaminergic dysfunction. Preclinical studies to determine whether peripherally administered CCK-8 or its analogs exert centrally mediated pharmacologic effects have yielded seemingly conflicting results. On the one hand, we have observed that systemically administered CCK-8 selectively alters local rates of cerebral glucose utilization in a pattern resembling that produced by neuroleptics. The peripheral administration of CCK-8 also induces various behavioral alterations including the suppression of signaled and Sidman avoidance learning as well as operant lever pressing in water deprived rats, and the inhibition of apomorphine induced stereotyped movements and contralateral turning in rats with unilateral mesencephalic lesions. Other results, however, cast doubt on the possibility that peripherally administered CCK-8 influences dopamine mediated functions within the central nervous system. We have, for example, been unable to demonstrate any significant alteration in ex vivo ^{125}I-CCK33 binding or in regional dopamine metabolism following acute peripheral injections of CCK-8. Moreover, our behavioral studies have indicated that the systemic administration of CCK-8 more potently inhibits water reinforced operant responding than central administration, and that these suppressive effects are significantly reduced by abdominal vagotomy. Finally, CCK-8 suppression of apomorphine induced contralateral turning in rats with unilateral mesencephalic lesions is also substantially reduced by abdominal vagotomy. The foregoing observations suggest that certain of the centrally mediated pharmacologic effects of peripherally administered CCK-8 reflect the stimulation of CCK receptors on vagal afferents to the nucleus tractus solitarius.

4. Therapeutic trials of cholecystokinin analogs.

The preclinical observations just described have prompted attempts to stimulate central CCK mediated functions in non-vagotomized patients who evidence disorders associated with dopamine system dysfunction. Parkinsonian patients optimally treated with either oral or intravenous levodopa received the CCK-8 analog, caerulein, by intramuscular injection. No significant alteration in motor function occurred. Similarly negative results were also obtained from a recently completed clinical trial of caerulein in neuroleptic-free schizophrenic patients. The therapeutic efficacy of caerulein in both these studies may have been limited by the relatively small doses possible to administer without significant gastrointestinal effects, doses which on a body weight basis range substantially below those required for behavioral activity in the experimental animal. On the other hand, the limited ability of CCK-8 or caerulein to penetrate the blood brain barrier as well as the relatively short plasma half life we found for these substances in man cast doubt on the ultimate clinical usefulness of CCK related peptides in the treatment of neuropsychiatric disease.

5. Development of Cholecystokinomimetics.

In an attempt to find alternative strategies for the pharmacologic manipulation of central CCK-8 mediated transmission, preclinical studies have continued to focus on the development of drugs to inhibit the inactivation of synaptically released CCK-8. Research conducted during the past year has confirmed our earlier observation that CCK-8 (CCK_{26-33}) is initially cleaved intrasynaptically at the Met_{28}-Gly_{29} bond, with subsequent breakdown to produce CCK_{31-33} and CCK_{32-33}. No regional differences in the products of CCK-8 degradation were found, although overall proteolysis rates were higher in striatum than in cortex. The effects of divalent metal cations and chelating agents on CCK-8 degradation indicate that the enzyme requires magnesium or manganese; its inhibition by zinc, cobalt, and PCMB suggests involvement of sulphydryl groups at or near the active site. Because of its magnesium dependency, the characteristics of the CCK degrading enzyme do not resemble those of other characterized membrane-bound metalloendopeptidases with similar specificity. Inhibitors directed against this enzyme are currently being synthesized in the laboratory and tested for their ability to block CCK-8 degradation as well as penetrate the blood brain barrier.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02263-09 ET

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical and Pharmacological Studies of Dopamine Receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

John W. Kebabian, Chief, Biochemical Neuropharmacology Section, ETB, NINCDS

T. Aqui, Visiting Fellow, Y. Furuki, Visiting Fellow, T. Yamamoto, Visiting Fellow, E. Frey, Sr. Staff Fellow, J.C. vanOene, Guest Researcher, A. Sidhu, Visiting Fellow, S. Guild, Visiting Fellow, S. Pocotte, Staff Fellow, ETB, NINCDS; T. Reisine, Staff Fellow, LCB, ADAMAHA; J. Neumeyer, Northeastern U.; C. Kaiser, SK&F Labs.; M. Caron, Duke University

COOPERATING UNITS *(if any)*

Laboratory of Cell Biology, NIMH, ADAMAHA

LAB/BRANCH

Experimental Therapeutics Branch

SECTION

Biochemical Neuropharmacology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
9.5	9.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The pharmacology of dopamine receptors was investigated with biochemical techniques during FY 85. The 'two dopamine receptor' hypothesis (which was formulated within ETB during 1979) provided the basis for these investigations. The BI Section developed an iodinated ligand which selectively interacts with the D-1 receptor. The development of this ligand permitted binding studies of the D-1 receptor to be performed with minute samples of tissue. The ligand permits the affinity of drugs for the D-1 receptor to be directly demonstrated (the binding studies provide only indirect evidence for the efficacy of drugs towards the receptor). The ligand also permits biochemical studies of the dopamine recognition site of the D-1 receptor to be undertaken. During FY 85, the D-2 dopamine receptor was also investigated. The process of 'desensitization' of the D-2 receptor in the intermediate lobe of the rat pituitary gland was investigated with an iodinated derivative of spiroperidol. Exposure of intermediate lobe tissue to D-2 agonists causes a diminution in the affinity of the receptor for antagonists and agonists. The drug-induced alterations in the properties of the binding site are correlated with drug-induced changes in the properties of the adenylate cyclase (which were described within the BI Section during FY 84).

The role of cAMP in the process of calcium-dependent hormone secretion was also investigated during FY 85. Studies were completed with the prolactin-secreting 7315c tumor cell. In this tumor, it was possible to demonstrate potentiation by forskolin, a non-specific activator of adenylate cyclase, of prolactin release from the cells induced by either potassium or ionomycin. Forskolin did not affect the rise in cytosolic calcium produced by either secretagogue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02578-03 ET

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacology and Cellular Biology of Peptidergic Neurons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Thomas L. O'Donohue, Head, Neuroendocrinology Unit, ETB, IRP, NINCDS

Stephen H. Buck, Thomas N. Chase, Bibie M. Chronwall, Patricia C. Contreras, John M. Farah, Barry Hoffer, V. John Massari, Gregory P. Mueller, Terry W. Moody, Remi Quirion, Kenner C. Rice, James L. Roberts, Carol A. Tamminga, Richard E. Tessel, Cinda J. Helke

COOPERATING UNITS *(if any)*

NIGMS PRAT, NIADDK LC, NIADDK DDB, NHLBI LC, Uniformed Services University of the Health Sciences (USUHS), University of Maryland, Columbia University, George Washington University, McGill University, University of Colorado

LAB/BRANCH

Experimental Therapeutics Branch

SECTION

Neuroendocrinology Unit

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
11.3	8.5	2.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to develop an understanding of the basic regulatory mechanisms in neurons and endocrine cells which secrete peptides, and through this understanding, develop novel pharmacotherapeutic approaches and agents for manipulating peptidergic neuronal and endocrine systems. Two projects are ongoing. The first studies the regulation of biosynthesis of peptides. The primary model investigated is the neuronal and endocrine opioid system which secretes ACTH, α-MSH and β-endorphin- peptides all derived from a common prohormone, pro-opiomelanocortin (POMC). Our investigations indicate that regulation of POMC gene expression occurs primarily at the transcriptional level. In addition, there appears to be coordinate regulation of the POMC gene and certain of the processing enzyme genes. The expression of POMC and processing enzyme genes appear to be regulated by cAMP-protein kinase A and diacylglycerol-protein kinase C mediated mechanisms.

The second investigation is focused on studies of the phencyclidine (PCP)/sigma opioid receptor and an endogenous ligand, α-endopsychosin, which interacts with these receptors. α-Endopsychosin was isolated from extracts of porcine brain based on the ability of the compound to inhibit the binding of phencyclidine to brain receptors. Structural studies indicate the α-endopsychosin is a peptide 26 amino acids long with a blocked amino terminus. Pharmacological studies of the peptide and PCP/sigma opioid analogues indicate that there are multiple PCP/sigma opioid receptors. Studies using the recently developed PCP acylating agent, Metaphit, also demonstrated that this compound would only antagonize the psycotomimetic effects of certain sigma opioids. Its ability to antagonize the effects of PCP suggest that Metaphit or a structural derivitive may find clinical utility for the treatment of PCP overdose.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02139-11 ET

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacology and Physiology of the Substantia Nigra and Basal Ganglia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Judith R. Walters, Ph.D., Chief, Physiological Neuropharmacology Section,
Experimental Therapeutics Branch, NINCDS

Debra Bergstrom, Ph.D., Thomas H. Lanthorn, Ph.D.,
Experimental Therapeutics Branch, NINCDS

Barton Weick, PRAT, NIGMS

COOPERATING UNITS *(if any)*

Pharmacology Section, Experimental Therapeutics Branch, NINCDS

LAB/BRANCH

Experimental Therapeutics Branch

SECTION

Physiological Neuropharmacology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	4	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1) Structure and Function of Dopamine Receptors. To better understand and define the dopamine receptors mediating the effects of dopamine agonists in the basal ganglia, potency series have been determined for a series of selective and nonselective dopamine agonists at dopamine autoreceptors and postsynaptic receptors. The results support the idea that the dopamine autoreceptors, located on the substantia nigra pars compacta dopamine neurons, are of the classic D-2 subtype. However, at least some of the postsynaptic dopamine receptors mediating haloperidol-reversible dopamine agonist effects on globus pallidus and substantia nigra cell activity appear to be relatively insensitive not only to the selective D-1 agonist, but to the selective D-2 dopamine agonists as well. This suggests that these receptors are not classic D-2, or D-1, receptors. In addition, there appears to be an interaction between the receptors mediating the effects of the nonselective dopamine agonists and the D-1 receptors; the consequences of stimulating the former is attenuated by a D-1 receptor antagonist.

2) Substantia Nigra Pars Reticulata in Epilepsy. It has been suggested that enhancement of GABAergic transmission within the substantia nigra prevents the motor manifestations of kindled seizures. We have recorded single unit activity of substantia nigra neurons during electrical seizures in components of the EEG, indicating that the substantia nigra is directly transmitting seizure activity in the kindled rat. To explore further the possible importance of this area as a therapeutic target in epilepsy, we have evaluated the effects of a diverse group of anticonvulsant drugs on these cells. Consistent inhibitory effects on tonic activity were produced only by drugs with proposed GABAergic mechanisms.

3) Glutamate and Related Neurotransmitter in the Substantia Nigra. At least 2 and perhaps 3 excitatory amino acid receptor types have been demonstrated on both the substantia nigra dopamine and pars reticulata cells neuron. Moreover, N-methyl-D-aspartate preferring receptors on the dopamine neurons mediate a previously undescribed firing pattern for these cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02265-09 ET

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Pharmacology, Biochemistry and Physiology of Central Neurotransmitters

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
Thomas N. Chase, M.D., Chief, Pharmacology Section, Experimental Therapeutics Branch, NINCDS

C. Shults, J. Juncos, L. Steardo, G. Bruno, P. Barone, C. Seratti, G. Fabbrini, C. Tamminga.

COOPERATING UNITS (if any)
Department of Psychiatry, Univ. of Maryland; Dept. of Psychiatry, Karolinska Institute; Dept. of Psychology, Bloomsburg University; Tissue Research Center, Harvard University

LAB/BRANCH
Experimental Therapeutics Branch, IRP, NINCDS

SECTION
Pharmacology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS: 7.5	PROFESSIONAL: 6.0	OTHER: 1.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to develop improved pharmaceutical therapies for central nervous system disease based on the relation between transmitter mechanisms and clinical function. Investigations focus on the dopamine system and closely interactive neural pathways as they relate to dementing and extrapyramidal disorders.

The search for transmitter abnormalities which serve as critical determinants for Alzheimer dementia has yielded evidence casting doubt on the importance of cholinergic system abnormalities: choline acetyltransferase reductions were no greater in the parietal association cortex, where positron emission tomography shows maximum cortical dysfunction, than in the relatively spared frontal lobe. Moreover, treatment with maximum tolerated doses of a potent muscarinic agonist failed to improve cognitive performance. On the other hand, somatostatin levels were mainly reduced in the posterior parietal cortex. Neuropeptide Y, which is partially co-localized with somatostatin, was not abnormal in any cortical area.

Motor fluctuations, which ultimately occur in most levodopa treated parkinsonian patients, probably do not reflect changes in peripheral clearance mechanisms but rather drug induced alterations in central pharmacodynamic factors. Both mechanical (wearable infusion pumps) and pharmaceutical (sustained release formulations) means for stabilizing plasma dopa levels are proving effective in attenuating these response fluctuations.

Many of the centrally mediated pharmacological effects of peripherally administered cholecystokinin-octapeptide (CCK-8) and related peptides in rodents now appear to reflect stimulation of vagal afferents. The peripheral injection of CCK-8 analogs has, nevertheless, proven therapeutically ineffective in nonvagotomized patients. Alternate strategies for influencing central cholecystokininergic mechanisms are thus being explored, including the development of relatively lipophilic compounds to inhibit the metaloendopeptidase found reponsible for initial CCK-8 degradation.

TAB 16 -- INFECTIOUS DISEASES BRANCH -- (IDB)

ANNUAL REPORT

October 1, 1984 through September 30, 1985

Infectious Diseases Branch

National Institute of Neurological and Communicative Diseases and Stroke

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ANNUAL REPORT

October 1, 1984 through September 30, 1985
Infectious Diseases Branch, IRP
National Institute of Neurological and
Communicative Disorders and Stroke

John Louis Sever, M.D., Ph.D., Chief

I. RESPONSIBILITY OF THE BRANCH

The responsibility of the Infectious Diseases Branch is to carry out coordinated research programs concerned with infections which damage the human nervous system. The Branch is divided into three sections: 1) Immunochemistry and Clinical Investigations (ICI); 2) Experimental Pathology (EP); and 3) Neurovirology and Molecular Virology (NMV). These sections utilize the techniques of immunology, clinical investigations including human volunteers and clinical trials, experimental pathology with small laboratory animals and nonhuman primates, virology, recombinant DNA technology and gene expression, tissue culture, and electron microscopy.

II. PROGRAM SEGMENTS

The program segments are: a) perinatal; b) acute; and c) chronic. In each segment we are concerned with: 1) etiology and diagnosis; 2) mechanisms of pathogenesis; 3) treatment; and 4) prevention.

III. RESEARCH AREAS

The present research areas in the program segments include:

A. Perinatal

Investigate methods for the early diagnosis of infections which damage the CNS and study mechanism of pathogenesis and prevention. Studies include herpes simplex, cytomegalovirus, Border disease and toxoplasmosis.

B. Acute

Investigate agents which may be responsible for acute neurological diseases. Current studies relate to Reye's syndrome and varicella-zoster.

C. Chronic

Study chronic neurological diseases using combined immunological, clinical, serological, virological, genetic, and electron microscopic approaches for possible infectious etiologies and mechanisms of pathogenesis. Whenever possible, explore methods for early diagnosis, treatment and prevention. Studies include subacute sclerosing panencephalitis, progressive multifocal leukoencephalitis, post polio muscular atrophy, amyotrophic lateral sclerosis, multiple sclerosis, AIDS and SAIDS.

III. SECTION ACTIVITIES

A. Section On Immunochemistry and Clinical Investigations (ICI)

1. Perinatal

a. Early Diagnosis

We have developed a new test for rapid detection of herpes simplex virus (HSV) using sensitive biotin-avidin reagents. The test involves capturing the virus on polystyrene coated with rabbit anti-HSV antibody. Biotin-linked anti-herpes antibody is used as the second antibody. The HSV antigen captured in such a double antibody sandwich is detected by reaction with streptavidin-alkaline phosphatase conjugate. With clinical specimens the test has a sensitivity of 95.6% and a specificity of 91.4% compared to the tissue culture method for detection of HSV. HSV antigen can be detected after virus infectivity was lost. This method is quite sensitive and specific compared to other non-tissue culture, direct assay methods. We are currently developing rapid tests for detection of cytomegalovirus (CMV).

b. Cytomegalovirus

Studies on children with congenital CMV infection are in progress. We have tested a child with severe neurological involvement following congenital cytomegalovirus infection. The child continues to excrete large amounts of virus in the urine and has significant T-helper and T-suppressor alterations and abnormal cellular and humoral responses to CMV, although responses to other antigens are normal. We have studied hospital personnel and teachers in special education schools who are exposed to persons excreting high levels of CMV. Our studies show that CMV is not airborne transmitted and that simple hygenic practices such as handwashing are sufficient to prevent spread of CMV among these individuals.

c. Border Disease

Border Disease (BD) is an animal model for viral induced dysmyelination and teratogenesis. We have developed primary cell cultures derived from control fetal kand adult ovine tissues and from fetal tissues from a lamb with BD. These cell cultures include CNS mixed glial, oligodendroglial enriched, dorsal root ganglia, sciatic nerve, leptomeningeal, choroid plexus, and peripheral white blood cells enriched for lymphocytes and monocytes. The cellular composition of these cultures were determined by immmunocytochemical methods. All cell types in tissue culture from both fetal and adult ovine tissues were found to be susceptible to BD viral infection. In light of our previous in situ studies of viral tophism in the persistently infected lambs, these tissue culture results suggest that the restricted replication of BD virus noted in the animal studies is not due solely to viral trophism for precurser cells. These data suggest that the type of cell infected may not be as important as the way in which BD virus affects the cells it infects.

In preparation for electron microscopic localization of BD viral proteins in neural cells, the techniques have been developed to visualize the DB virion which is spherical and 70 nm in diameter. Also, the methodology has been developed to prepare infected neural cells where cellular morphology, BD viral and neural cell antigenicity are maintained. From this work the conclusions are that BD viral proteins are intracellular and associated primarily with membranes.

d. Collaborative Study - Toxoplasmosis

For the Collaborative Perinatal Study, we are completing two major reports. The first paper is on Toxoplasmosis During Pregnancy and the second is a complete analysis of Maternal Infections in Abnormal and Matched Control Pregnancies. These studies will complete the "Core" investigations of this project. The clinical data and serum specimens are maintained as a national resource for NIH and outside groups. Proposals for use of the data are reviewed by a NINCDS committee and when approved, the information and specimens are released. Clinical data is on six computer tapes and the serial serum specimen from 58,000 pregnancies are in the Serum Center of the IDB. At present we are supplying specimens for studies being conducted by the NICHD, NIAID, University of California and the CDC.

2. Acute

Studies are being conducted using monkeys with simian varicella as a model for human varicella and possibly Reye's Syndrome (With the EP Section).

3. Chronic

a. MS, SSPE, EAE and Coronavirus Demyelination

We continue in our efforts to define the role of etiological agents and host responses in neurological diseases. In our studies of multiple sclerosis (MS), subacute sclerosing panencephalitis (SSPE), and other neurological diseases, we have found that a new silver staining technique for protein in acrylamide gel electrophoresis to detect oligoclonal bands in the CSF increases the sensitivity of the test greatly. This increased sensitivity gives us the ability to look for additional proteins which are related to the disease process. In studies of experimental allergic encephalitis (EAE) in monkeys, we demonstrated that oligoclonal bands also can be detected in the CSF. In measles antibody positive animals there was no change in the measles antibody level during the course of the disease nor did measles antibody appear in the CSF or oligoclonal bands. Animal models of chronic CNS infection (coronaviruses) and autoimmunity (EAE) have been used in studies of possible therapeutic materials for MS. Acute and subacute viral demyelination was produced in mice by injecting the hepatotropic strain of mouse coronavirus intracerebrally into C_3H mice. The animals developed a predictable clinical syndrome and specimens were studied for virus recovery, serological response and histopathology. EAE disease in the guinea pig model was used to study the effect of drugs in preventing development and progression of disease. Thymosin fraction 5 which has been suggested as a treatment for MS was studied. None of the 3 doses used were effective in reducing the incidence or severity of EAE. Therefore, this drug does not seem to be highly useful in prevention of this disease.

b. SAIDS And AIDS

Cellular and humoral immunological studies of Simian Acquired Immunodeficiency Syndrome (SAIDS), Aquired Immunodeficiency Syndrome (AIDS), and Subacute Sclerosing Panencephalitis (SSPE) are being conducted. In SAIDS, the monoclonal OKT3 antigen has not been found to be present on either normal or SAIDS infected lymphocytes. The NEN-Lyt 9.6 monoclonal antibody was found to be a better reagent than OKT-11 in detecting the E-rosseting marker on lymphocytes. These findings will be applicable to all other studies in which lymphocyte markers are needed to investigate immunological diseases in nonhuman primates. Neutropenia occurred early in the SAIDS monkeys. The inversion of OKT4/OKT8 ratios is not found in SAIDS infected animals. Severe hypogammaglobulinemia and decreased lymphocyte mitogen responses appear in the later stages of SAIDS. Studies are in progress to determine the retrovirus susceptible cells which lead to immunosuppression and SAIDS. Patients with SSPE, a chronic measles infection of the brain, are being investigated for abnormalities in OKT4/OKT8 ratios, complement subtype deficiencies, complement haplotype markers, immune complexes and _in situ_ hybridization studies for measles genome in peripheral blood lymphocytes. Also included are _in vitro_ studies of cellular and humoral immune functions with autologous measles infected lymphocytes.

c. PPMA, ALS, PML - Clinical and Laboratory Studies

Clinical, immunological and virological studies of late post poliomyelitis muscular atrophy (PPMA), ALS, PML, polyneuropathies, polymyositis and metabolic myopathies are being conducted. Patients with Progressive Multifocal Leukoencephalopathy are being studied prospectively for immune defects specific for the etiologic agent, JC virus, comparison of _in situ_ hybridization and antigen detection for diagnosis using brain biopsy, and a comparison of CT scanning and MRI scanning as possible diagnostic procedures.

We have performed several clinical, immunological and virological studies involving patients with neuromuscular and demyelinating diseases. Specifically, we have defined the clinical spectrum of new symptoms and signs that occur many years later in patients with prior paralytic poliomyelitis. We have also completed a 12 year follow-up study of patients with new symptoms which established the rate of progression on a year to year basis. The pathogenetic mechanisms of this disease were investigated with a) a detailed virological and immunological screening in the serum and CSF, b) histological studies in their newly affected muscles, c) electrophysiological investigation including single fiber EMG, and d) epidemiological survey of 2,000 previously affected patients to establish the frequency of the disease. We found that in post-polio patients there appears to be peripheral disintegration of the distal axons of the surviving motor neurons. The status of the upper motor neurons in post-polio patients was also studied using PET scan and ^{18}F-2-deoxy-D glucose and the findings were compared with the pattern seen in ALS patients.

We observed that the metabolic activity of the motor sensory cortex is normal in post-polio motor neuron diseases whereas in ALS patients there appears to be widespread hypometabolism involving the motor and paramotor cortical regions. We have also completed an experimental therapeutic trial in ALS patients using recombinant DNA interferon. This was ineffective in arresting the progression of the disease or changing the metabolic status of the motor cortex, as studied with the PET scan.

We have also studied immunologically, immunocytochemically, and neurophysiologically patients with peripheral neuropathies. We have identified that the monoclonal IgM from patients with paraproteinemic neuropathies is an antibody against either the myelin associated glycoprotein (MAG) or against different glycolipids and gangliosides, the identity of which was defined. Thus, glycolipids can be new, strong antigens in the pathogenesis of patients with neuropathy. Using immunochemical techniques we have also determined the nature of amyloid protein in patients with aporadic amyloid polyneuropathy as being related to immunoglobulin light chain. In another group of 14 de-afferented patients with ataxic sensory neuropathy but normal strength, we studied the mechanism and importance of proprioceptive input for the motor control.

Investigating for mechanisms of demyelination in the CNS and seeking possible interaction of myelin supporting cells with cells of the lymphoid organs, we found that the thymic hormone thymosin beta 4 is a shared antigen between human oligodendrocytes and macrophages or other $1a^+$ cells of the lymphoid system. This supports the presence of an immune link between activated macrophages and oligodendrocytes, which can help us to understand the mechanism of destruction of oligodendrocytes in human immune demyelinating diseases of the CNS.

Other studies of neuromuscular diseases include a) the establishment of the viral model of polymyositis in monkeys using a well characterized (by IDB virologists) new retrovirus D, which provides evidence that the virus directly or via infected cells is responsible for the muscle damage, and b) the identification of a metabolic defect due to carnitine deficiency in the muscle of patients with cystinosis. The latter finding prompted an ongoing experimental therapeutic trial with carnitine in an effort to increase the strength of these patients.

B. Section On Experimental Pathology (EP)

1. Perinatal

Our work with the rhesus monkey model of Group B Streptococci (GBS) casts doubt on the efficacy of purposed polysaccaride vaccines for humans. We have shown that protection from GBS intraamniotic infection was not associated with maternal antibody titer, prior maternal immunization or dose of GBS administered. The efficacy of hyperimmune human IgG in treating GBS infected rhesus infants is now under study.

2. Acute

A new simian varicella has been studied in guinea pigs and several species of monkeys. SV virus produces mild disseminated chicken-pox like lesions in the rhesus monkey. More severe infection with occassional fatal outcome is seen in African monkeys. Guinea pigs inoculated via various routes showed no signs of infection. Studies are now in progress to determine if SV is able to establish a latent state in rhesus monkeys. If SV latency develops, a model for human varicella zoster infections in nonhuman primates will be available for further investigation. The model may also be of value for investigations of Reye's Syndrome.

3. Chronic

a. Neuro-oncogenic studies are being conducted with the owl monkeys inoculated intracerebrally with JC virus (MAD 1 and 4), human polymomavirus (With the NMV Section).

b. Studies of simian AIDS (SAIDS) are being conducted to determine the true cause of this disease. Specimens from AIDS patients are also being studied (With the NMV Section).

c. An investigation of the mechanism of clearance of chronic infection with SHF by super infection with a related virus is being conducted (With the NMV Section).

C. Section On Neurovirology & Molecular Virology (NMV)

1. Perinatal

A pilot study of SAIDS in newborn monkeys and fetuses has been initiated (With the EP Section).

2. Acute

Studies of the clearance of persistant SHF infections by acute virus strains are being conducted (With the EP Section).

3. Chronic

a. JC Virus - PML

We have established a human fetal glial cell line using an origin defective SV40 DNA which is susceptible to JC virus infection, demonstrates characteristics of astrocytes and synthesizes a replication proficient SV40 T protein. Comparing JC virus growth in this cell with primary human fetal glial cells, we have observed that both astroglial and oligodendroglial cells produce JC T protein, replicate JCV DNA and produce infectious virions. Both the cell line and primary cultures of human glial cells produce a p53 protein which complexes with the SV40 T protein but not with the JCV T protein. We have also demonstrated the presence of the JCV genome in fixed tissue sections from PML brain using biotin labeled JCV DNA and *in situ* hybridization. The molecular detection of viral genome copies in infected PML tissue directly correlates with the presence of viral antigen in oligodendroglial cells indicating that these cells are the main target for JCV growth. Bizarre astrocytes associated with the pathology of PML disease also show evidence for JCV DNA using *in situ* hybridization techniques. Some bizarre astrocytes also synthesize virion capsid proteins indicating that such cells may not be transformed but rather represent a distinctively altered state of infection which permits limited virus growth. In owl monkey brain, JCV does transform cells or causes tumors. One such tumor, an astrocytoma, was explanted, dissected to a cell suspension, inoculated into another owl monkey or planted in tissue culture. These cells caused a tumor in the recipient animal but failed to establish a cell line in culture. Integrated JCV genome and T protein have been identified in the tumor cells of the recipient owl monkey.

b. SHF

Differences between acute and persistent infections are being sought via use of the patas monkey - simian hemorrhagic fever virus model. Virological and immunological techniques are being used to determine the mechanism of elimination of persistent SHF virus infection by superinfection. Physical-chemical differences between acute and persistent strains of SHF virus are being sought by monoclonal antibody and molecular biology techniques. Cellular immunology techniques are being used to elucidate the cellular interactions involved in restricting the immune response and maintaining tolerance of persistent SHF virus infection. Immune enhancement leading to death is being studied in macaque monkeys (With the EP Section).

c. SAIDS

We and others have implicated a type D retrovirus related to Mason-Pfizer monkey virus (MPMV) as the etiologic agent of SAIDS. Recently a different retrovirus related antigenically and morphologically to HTLV-III, which has been called STLV-III, was isolated from such monkeys with immunosuppressive disease. Work is in progress to determine whether a similar agent can be isolated from our mocula which elicity SAIDS when injected into susceptible animals or from animals with SAIDS. The target cells and mechanisms of immunosuppression of SAIDS virus are also under study by immunological, virological and molecular biological techniques. Studies are also in progress to determine whether animals can be protected from fatal SAIDS by vaccinating them with attenuated strains of SAIDS retrovirus (With the EP Section).

Virological and immunological support is also provided for the AIDS studies being performed by investigators of the ICI Section.

IV. Findings

A Perinatal

1. Rapid Detection Of Herpes Simplex Virus

A new capture technique using biotin-streptavidin with the enzyme linked immunosorbent assay method was perfected for the rapid detection of herpes simplex virus. This test is positive in 4½ hours.

2. BD Virus Causes Dysmyelination and Microencephaly In Lambs

Studies with Border Disease (BD) virus demonstrated that congenital infection results in microencephaly associated with dysmyelination. BD ocurrs naturally in lambs in California.

3. Incidence Of Clinical Maternal Infections Determined For Collaborative Study

In the prospective study of 44,000 pregnant women, most women experienced one clinical infection during pregnancy (89.7%). The highest incidence of infection was for vaginitis, influenza/flu-like disease, and infections of the kidney, ureter and bladder.

4. Maternal Immunity To Group B Streptococci Does Not Protect The Fetus -In A monkey Model

Group B Streptococci were shown to grow well in amniotic fluid and maternal antibody did not protect the fetus from infection. These findings are important in relation to present attempts to develop a vaccine for GBS in humans.

B. Acute

1. IgM Antibody To Varicella Develops in 5-8 Days In Patas Monkeys

Studies in patas monkeys with simian varicella showed that viremia was present 3 days after infection; IgM abtibody appeared at 5-8 days and IgG antibody was present at 10-14 days.

C. Chronic

1. Microsticks Developed For Enzyme-linked Immunosorbent Assays

A new method using polycarbonate-coated microsticks as solid phase carriers was developed for ELISA tests. This method provides greatly improved uniformity and reproducibility of results.

2. Oligoclonal Bands Present In CSF of Monkeys With EAE

Monkeys with EAE were shown to develop oligoclonal IgG bands in the CSF at the same time that clinical signs of disease began to appear.

3. EAE In Guinea Pigs Not Suppressed By Thymosin Factor V

Experimental studies showed no suppression of EAE by Thymosin. This information must be considered in relation to proposed use of Thymosin for the treatment of MS.

4. Demyelination Induced By Mouse Hepatitis Virus

MHV strain A59 induced acute and subacute demyelinating disease in the CNS of C3H mice. Viral antigens persisted for up to 4 weeks. This provides a model for coronavirus induced demyelination.

5. Late Effects Of Poliomyelitis

The clinical syndrome of late postpoliomyelitis muscular atrophy (PPMA) have been described and possible pathogenetic mechanisms of the disease were investigated. A 12 year followup study is in progress.

6. Human Thymic Hormones Present In Certain Lymphocytes, Lymphoid Organs and Oligodendrocytes

The demonstration of receptors for human thymic hormones in certain lymphocytes, lymphoid organs and oligodendrocytes suggests a possible link between the lymphoid system and oligodendrocytes in the brain.

7. Identification Of New Antigens In The Peripheral Nerves Of Patients With Paraproteinemic Polyneuropathies

Monoclonal IgM antibody against peripheral nerve MAG, glycolipids and ganglioside was demonstrated in patients with paraproteinemic polyneuropathies. This appears to be important in the pathogenesis of the disease. The CSF and clinical findings in the disease were studied in detail.

8. Amyloid In Patients With Polyneuropathy And Hypernephroma Is Related To Immunoglobulin Light Chains

Although patients may show no plasma cell dyscrasia amyloid can be of immunoglobulin origin, perhaps produced by plasma cells in the tumor. These patients may now be treated with immunosuppression.

9. Children With Cystinosis And Renal Fanconi Syndrome Have Carnitine Deficiency In Plasma And Muscle

These findings have suggested that these children can be treated with carnitine. Clinical studies are in progress.

10. SAIDS Can Be Transmitted With Saliva Or Urine

Studies of saliva and urine from SAIDS monkeys showed the presence of virus. This virus could transmit the disease.

11. AIDS And SAIDS Viruses Have Similar Distinctive Morphologies

EM studies of AIDS and SAIDS showed similar mature virions with cylindrical cores. A model was developed to show the morphology. This is of assistance for identifying these viruses by EM.

12. Polymyositis Present In 50% Of SAIDS Animals - A Model For Polymyositis

SAIDS monkeys frequently develop polymyositis. Virus was isolated from the muscle and demonstrated by peroxidase and FA staining. The disease is similar to that seen in human polymyositis.

13. New Line Of Fetal Astroglial Cells Support JC Virus Growth

An immortalized human fetal astroglial cell line was developed which supports the growth of JC virus. This cell line provides a research source of human fetal brain cells.

14. Studies Of The Kinetics Of JC Virus Growth Indicate That More Cells Produce T Antigen Than Can Replicate The Viral DNA

This indicates that some human glial cells are able to synthesize only T protein while others replicate the viral DNA as well.

15. Both Human Oligodendroglial And Astroglial Cells Can Produce Infectious JC Virus

This shows that the infection is not limited to the myelin producing cells of the brain.

16. Human Fetal Glial Cells Synthesize A P-53 Protein

This protein complexes with the SV40 T protein but not with the JC virus T protein.

17. JC DNA Can Be Identified By In Situ Hybridization In Paraffin Embedded And Frozen PML Brain Tissues

This provides definitive evidence for the involvement of JC virus in the pathology of PML.

18. There Is A Direct Correlation Between The Detection Of The JC Genome In Oligodendrocytes Of PML Tissue And The Production Of JC Viral Capsid Antigen

This indicates that in infected cells both DNA replication and virion multiplication take place and that oligodendrocytes are the main target for JC virus infection.

19. Bizarre Astrocytes In PML Show Evidence Of JC Virus DNA Replication And Capsid Protein

This shows that bizarre astrocytes are not transformed by JC virus but rather could be "distinctively altered" and allow limited growth of JC.

20. Tumor Cells From A JC Induced Astrocytoma In An Owl Monkey Were Shown To Be Oncogenic In A Recipient Animal

This is the first observation of the tumorigenicity of virus induced brain tumor cells which may help establish a model for brain tumor experiments.

CONTRACT NARRATIVE

Infectious Diseases Branch, IRP, NINCDS

Fiscal Year 1985

Microbiological Associates: (N01-NS-3-2316)

TITLE: Development and Delivery of Antigen, Antisera, and Viral Diagnostic Reagents.

Contractor's Project Director: Dr. David A. Fuccillo

Current Funding: $350,000.00

Objectives: This is a service contract to provide research reagents for studies of neurological diseases which may have infectious etiologies and special investigations of polyomaviruses, AIDS and simian AIDS (SAIDS).

Major Findings: Viral diagnostic reagents have been provided for herpes viruses types I and II, cytomegalovirus, measles, rubella, influenza, and varicella. These antigens are used in an attempt to identify the etiology of neurological infections. Evaluation of reagents and materials required to produce successful enzyme-linked immunosorbent assays (ELISA) was accomplished. ELISA reagents have been developed to detect varicella infection in monkeys and to provide the reagents necessary to study varicella latency. Reagents for acquired immune deficiency syndrome (AIDS) are being developed to study this highly fatal disease. A similar outbreak of simian AIDS-like disease (SAIDS) has occurred in rhesus monkeys. Reagents to study rhesus monkey CMV and its relationship to SAIDS have been prepared. Large quantities of a retrovirus are being prepared for comparison studies to be done against a similar virus found in SAIDS. Reagents for ELISA tests have been developed for the JC papovavirus. Reagents have been prepared for studies on the molecular genetics of the BK and JC virus.

Significance to the NINCDS Program and Biomedical Research: This contract provides the Infectious Diseases Branch with reagents which are made under standard protocols and with similar cells and strains of viruses from one production lot to another. Many of the reports helped establish the frequency of disease during pregnancy, syndromes that develop and information on which to base rational therapeutic and preventive measures. Other studies relate to the possible infectious etiologies of various neurological diseases. The causative agent of AIDS is now considered to be a retrovirus (HTLV-III). An animal disease model such as SAIDS would greatly help in understanding its pathogenesis and neurological consequences.

Polyomavirus studies provide basic information as to the initiation of viral growth in brain tissue and eventual production of malignancy. These studies may help to explain the host-related mechanism of persistent infection for progressive multifocal leukoencephalopathy (PML) and other slow viral infections.

Proposed Course: The contract will be continued for the next year but at a significantly reduced rate.

Publications:

Iltis, J.P., Achilli, G., Madden, D.L., Sever, J.L. Serologic study by enzyme-linked immunosorbent assay of the IgM antibody response in the patas monkey following experimental simian varicella virus infection. Diagnostic Immunology 2:137-142, 1984.

Achilli, G., Sarasini, A., Revello, M.G., Torsellini-gerna, M., Gerna, G., Iltis, J.P., Madden, D.L. Kinetics of virus-specific IgG and IgM antibody response in patas monkeys experimentally infected with delta herpesvirus by enzyme-linked immunosorbent assay. Microbiologica 7:287-297, 1984.

Achilli, G., Sarasini, A., Gerna, G., Iltis, J.P., Madden, D.L. Antibody response of patas monkeys to experimental infection with delta herpesvirus. Eur. J. Clin. Microbiol. 3(2):158-159, 1984.

Shekarchi, I.C., Tzan, N., Sever, J.L., Madden, D.L. Polycarbonate-coated microsticks as solid-phase carriers in an enzyme-linked immunosorbent assay for rubella antibody. J. Clin. Microbiol. 21(1):92-96, 1985.

CONTRACT NARRATIVE

Infectious Diseases Branch, IRP, NINCDS

Fiscal Year 1985

Meloy Laboratories, Inc.: (NO1-NS-5-2377)

Title: Isolated Housing and Care of Animals Used in Studies of Infectious Diseases of the Central Nervous System.

Contractor's Project Director: Dr. Jere M. Phillips

Date Contract Initiated: 16 May 1985

Current Annual Level: $251,794.00

Objectives: The contract provides isolated housing and care for laboratory rodents and a colony of nonhuman primates consisting of several genera. The animals are on experimental studies directed by written protocols. They require monitoring daily for clinical signs of disease. Biological specimens are collected as prescribed by protocols. The aims of the contract are to provide the facilities which permit animal studies that are judiciously planned to be humanely carried out. Animal studies carried out for the IDB, NINCDS are designed to investigate the etiology, pathogenesis, early diagnosis, treatment and prevention of both known and suspected infectious diseases of the nervous system.

Methods Employed: Animals are quarantined, conditioned and screened for pre-existing antibodies to agents under investigation. Seronegative animals are inoculated by a variety of routes. The infected animals are then held in individual isolation units, monitored and tested as directed in written protocols.

Major Findings: This contract satisfactorily provides housing and care for most of the laboratory animals needed for research in the Infectious Diseases Branch. Animals are used in a number of studies of the infections of the central nervous system (CNS). Experimental animals who become sick are promptly identified and supportive therapy instituted. The investigators on the contract provide overall daily clinical care for the entire colony, with strict isolation procedures carried out at all times. The Contractor's Project Director makes modifications of studies when necessary to achieve the overall goals of the contract.

Significance to the NINCDS Program and Biomedical Research: The goal of the NINCDS is to carry out planned, directed research programs concerned with the diseases which effect the human nervous system. This contract provides an important source for housing and monitoring laboratory animal models used in the study of infectious neurological diseases. The facility is accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC) which insures proper and humane care at all times.

Proposed Course: This contract will be continued for the following year to provide the isolated housing and care of a colony of nonhuman primates and rodents inoculated with various infectious agents of the CNS.

Publications: None. All publications from this contract are listed in each section of the Infectious Diseases Branch Annual Report.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-00402-29-ID

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Perinatal Infections Causing Damage to the Children in the CPP

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

John L. Sever	Chief	IDB, IRP, NINCDS
David L. Madden	Veterinary Director	IDB, IRP, NINCDS
Other: Jonas Ellenberg	Deputy Chief	OB & FS, OD, NINCDS
Nancy Tzan	Microbiologist	IDB, IRP, NINCDS
Dorothy M. Edmonds	Clinical Nurse	IDB, IRP, NINCDS

COOPERATING UNITS (if any)

OB & FS, OD, NINCDS

LAB/BRANCH

Infectious Diseases Branch

SECTION

Immunochemistry and Clinical Investigations

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this study is to determine insofar as possible the role of perinatal infections in the production of fetal damage. To accomplish this, clinical data and a large number of serial serum specimens were obtained from the 58,000 women and their children in the Collaborative Perinatal Project. A number of reports and publications have come from the study. During this year papers have been published summarizing approaches used by the study and the incidence of clinical infections in the study population. Current efforts have been focused on completing the analysis and publication of the two remaining major studies from the project: 1) Toxoplasmosis and Fetal Damage and 2) The Study Of The Pregnancies Of Abnormal Children And Matched Controls. We are also supplying clinical data and serum specimens from the Collaborative Project for several other studies including: NICHD Study On Maternal Diabetes; NIAID Investigations Of AIDS; University of California, Berkeley studies of cancer and thyroid disease; and a new study with the CDC on fetal abnormalities caused by parvovirus infections.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-NS-02532-03-ID

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Study of AIDS and SAIDS Neurological Findings and Etiology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	John L. Sever	Chief,	IDB, IRP, NINCDS
Others:	Sidney A. Houff	Neurologist	IDB, IRP, NINCDS
	William T. London	Veterinary Director	IDB, IRP, NINCDS
	Maneth Gravell	Research Microbiologist	IDB, IRP, NINCDS
	David L. Madden	Veterinary Director	IDB, IRP, NINCDS
	Lata Nerurkar	Special Expert	IDB, IRP, NINCDS
	Delia Budzko	Special Expert	IDB, IRP, NINCDS
	Marinos Dalakas	Senior Staff Fellow	IDB, IRP, NINCDS
	Barbara J. Potts	Staff Fellow	IDB, IRP, NINCDS
	Gail Scherba	Staff Fellow	IDB, IRP, NINCDS
	Marta Monzon	Visiting Associate	IDB, IRP, NINCDS

COOPERATING UNITS (if any)
California Primate Research Center, Davis, CA; Drs. Henry Masur and Abe Macher, Department of Critical Care Medicine, CC, NIH; Dr. Gopal Murti, St. Jude Children's Research Hospital, Memphis, TN.

LAB/BRANCH
Infectious Diseases Branch

SECTION
Unit on Clinical Investigations

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
13.80	6.25	7.55

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Clinical and laboratory studies were conducted to determine the etiological agents and neurological manifestations of acquired immunodeficiency syndromes in man (AIDS) and in nonhuman primates (SAIDS). Patients with neurological complications of AIDS have been admitted to the Neurology Service of the NIH Clinical Center for study. Patients admitted to other Institutes have been seen by the Infectious Diseases Branch Consultation Service. Patients have been evaluated to determine the spectrum of neurological illnesses found in AIDS. Appropriate virological and immunological studies are being conducted by IDB and collaborating laboratories. Retrovirus-like particles haves been demonstrated in testes, salivary gland and prostate of patients with AIDS.

Pronounced neutropenia has been detected in animals in the early stages of SAIDS with generalized immunosuppression occurring later. Isolations of SAIDS D retrovirus have been made from saliva and urine of diseased animals and SAIDS has been experimentally transmitted to a susceptible animal with an isolate from urine. Kaposi's sarcoma has also been successfully transmitted to rhesus monkeys with homogenates of tissues from animals with SAIDS.

About 50% of our animals with SAIDS have developed polymyositis with many of the characteristics of viral-induced polymyositis of humans. SAIDS retrovirus has been shown to be present in and has been isolated from involved muscles. Studies of polymyositis associated with SAIDS retrovirus infections should provide a highly reproducible animal model which may further our understanding of human polymyositis.

A morphological model has been developed to explain the different structures seen when AIDS and SAIDS retroviruses are thin sectioned and observed by electron microscopy.

Findings from studies of nonhuman primates with SAIDS are being compared with those obtained through our AIDS protocols.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-NS-01985-14-ID

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Viral and Nonviral Antigens or Antibodies in Perinatal and Neurological Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: David L. Madden,	Veterinary Director,	IDB, IRP, NINCDS
John L. Sever	Chief	IDB, IRP, NINCDS
Lata Nerurkar	Special Expert	IDB, IRP, NINCDS
Delia Budzko	Special Expert	IDB, IRP, NINCDS
Mary Ann South	Guest Researcher	IDB, IRP, NINCDS

COOPERATING UNITS *(if any)*
Microbiological Associates, Inc.

LAB/BRANCH
Infectious Diseases Branch

SECTION
Immunochemistry and Clinical Investigations

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.75	.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Techniques to improve methods for rapid viral diagnosis of acute and persistent infections which affect the CNS continue to receive special emphasis. The use of short term tissue culture technique (24 hours followed by staining of cells using an anti-herpes antibody linked to biotin and a fluorescent labeled avidin conjugate) was shown to be a highly efficient system for detecting herpes antigen. We have also developed a capture technique to identify viral antigen directly in specimens without tissue culture. Comparison of this test with culture techniques indicate that non-infectious antigen can be detected for periods of time after viable virus has disappeared. Studies of a child with severe neurological involvement following congenital cytomegalovirus continue. The child has significant T helper and suppressor ratio alterations and abnormal cellular and humoral responses to CMV antigen; the immunological responses to other viral and non specific mitogens have been normal. We found the use of silver staining technique for detection of oligoclonal bands in MS was more sensitive than Coomasie blue staining. We have developed a highly reproducible animal model for the study of acute and subacute demyelinating neurological disease using coronavirus and a strain of mice that is genetically resistent to mouse hepatitis virus infection. We found that thymosin had no suppressive effect on the incidence or severity of experimental allergic encephalomyelitis (EAE) in guinea pigs. We have examined the humoral and cellular immune responses of patients with SSPE. Patients with SSPE have no detectable helper/suppressor abnormalities nor abnormalities in the non specific mitogen proliferative response during progressive disease status. No IgM was demonstrated in serum against measles virus. However, the IgG was significantly elevated. Increases in the proportion of IgG_1 and IgG_4 were observed in the IgG component of serum.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02038-13-ID

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Combined Clinical, Viral and Immunological Studies of Peripheral and CNS Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Marinos C. Dalakas, Senior Staff Fellow, IDB, IRP, NINCDS

John L. Sever,	Chief,	IDB, IRP, NINCDS
David L. Madden	Veterinary Director	IDB, IRP, NINCDS
Maneth Gravell	Research Microbiologist	IDB, IRP, NINCDS
Sidney A. Houff	Neurologist	IDB, IRP, NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Infectious Diseases Branch

SECTION

Immunochemistry and Clinical Investigations

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Clinical and laboratory studies are conducted to determine etiology (infection, immunity and/or genetics) for chronic diseases of the peripheral and central nervous system. Current studies include amyotrophic lateral sclerosis, (ALS), polymyositis/dermatomyositis, demyelinating polyneuropathies, progressive multifocal leukoencephalopathy, and myasthenia gravis. Combined clinical data, genetic information, HLA and MLC typing and studies of virus serology and virus isolation are performed. The nature of oligoclonal bands found in the CSF of patients with chronic neurological diseases is under investigation. A neuromuscular disease that occurs in patients who have had poliomyelitis at an early age has been clinically defined; the possibility that this might be due to a late polio virus infection or an abnormal immunoregulation and an immune reaction to neuronal cells is under investigation. IgM monoclonal band has been identified in the spinal fluid of patients with paraproteinemic polyneuropathies and an abnormal blood-CSF was found. The pathogenetic mechanisms of patients with a chronic, sensory, "ataxic" neuropathy were examined and the role of proprioceptive afferent inputs for their postural maintenance was investigated. The metabolic activity of the cortex in ALS patients is being studied using the PET scan and ^{18}F 2-deoxy-D-glucose; hypometabolism was demonstrated not only in the motor but also in the paramotor and sensory cortex, suggesting that ALS is a rather generalized process affecting many cortical regions. The effect of aging on the neuromuscular systems is being investigated electrophysiologically and morphologically, in the muscle and nerve biopsies of normal elderly patients and patients with Alzheimer's disease. Muscle biopsies from patients with nephropathic cystinosis and renal Fanconi syndrome were studied morphologically and biochemically. Signs of a metabolic lipid storage myopathy due to carnitine deficiency were found; this prompted us to start a therapeutic study with carnitine replacement.

Z01-NS-02038-13-ID

Gregory Elder	Medical Staff Fellow	IDB, IRP, NINCDS
Allen Aksamit	Medical Staff Fellow	IDB, IRP, NINCDS
Giovanni DiChiro	Neuroradiologist	SNB, IRP, NINCDS
Bruce Trapp	Senior Staff Fellow	IDB, IRP, NINCDS
Nick Papadopoulos	Biochemist	CC, NIH
Jerry Sanes	Neurophysiologist	NIMH
David Fuccillo,	Project Director, Virology,	Micro. Biol. Assoc., Bethesda, Md.
Neil Cutler	Chief,	LNS, IRP, NIA
Paul Plotz	Rheumatologist	ARB, IRP, NIADDK
William Gahl	Chief,	HGB, IRP, NICHHD
Mark Hallet	Clinical Director,	NINCDS
Richard Quarles	Biochemist	DMN, IRP, NINCDS

READEX FICHE

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02020-11 ID

October 1, 1984 through September 30, 1985

[illegible]

[illegible]

Infectious Diseases Branch

Immunochemistry and Clinical Investigations

NINCDS, NIH, Bethesda, Maryland 20205

1.5	1	0.5

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

Clinical and laboratory studies are conducted to determine etiology (infection, immunity and/or genetic) for chronic diseases of the peripheral and central nervous system. Current studies include amyotrophic lateral sclerosis (ALS), polymyositis/dermatomyositis, demyelinating polyneuropathies, progressive multifocal leukoencephalopathy, and myasthenia gravis. Detailed clinical data, genetic information, HLA and [illegible] typing and studies of virus serology and virus isolation are performed. The nature of oligoclonal bands found in the CSF of patients with chronic neurological diseases is under investigation. A neuromuscular disease that occurs in patients who have had poliomyelitis at an early age has been clinically defined; the possibility that this might be due to a late polio virus infection or an abnormal immunoregulation and an immune reaction to neuronal cells is under investigation. IgM oligoclonal band has been identified in the spinal fluid of patients with paraproteinemic polyneuropathies and an abnormal [illegible] was found. The pathogenetic mechanisms of patients with a [illegible] "ataxic" neuropathy were examined and the role of [illegible] afferent inputs for their postural maintenance was investigated. The metabolic activity of the cortex in ALS patients is being studied using the PET scan and [illegible] hypometabolism was demonstrated not only in the motor but also in the [illegible] and sensory cortex, suggesting that ALS is a rather generalized process affecting many cortical regions. The effect of aging on the neuromuscular systems is being investigated electrophysiologically and morphologically in the muscle and nerve biopsies of normal elderly patients and patients with Alzheimer's disease. Muscle biopsies from patients with nephropathic cystinosis and renal Fanconi syndrome were studied morphologically and biochemically. Signs of a metabolic lipid storage myopathy due to carnitine deficiency were found; this prompted us to start a therapeutic study with carnitine replacement.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-01731-17-ID

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Isolation, Characterization and Diagnosis of Infectious Agents from Chronic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Maneth Gravell	Research Microbiologist	IDB, IRP, NINCDS
Other:	Rebecca S. Hamilton	Biologist	IDB, IRP, NINCDS
	Marta Monzon	Virologist	Microbiological Associates, Inc.

COOPERATING UNITS (if any)

Section on Experimental Pathology, IDB, IRP, NINCDS

LAB/BRANCH

Infectious Diseases Branch

SECTION

Neurovirology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.2	.1	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Simian hemorrhagic fever (SHF) virus is an unclassified togavirus which resembles most closely the flaviviruses in its mode of replication. Four strains of SHF virus have been identified. Two of the strains produce persistent infections in patas monkeys and the others, acute infections. All strains produce acute infections of monkeys of the genus Macaca which are nearly always fatal.

Although SHF virus and flaviruses have many structural and morphogenetic similarities, they also differ considerably. By use of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), we have obtained data which indicate that SHF virions contain five polypeptides. Flaviviruses have been reported to contain only three. Furthermore, by use of enzyme-linked immunosorbent assay (ELISA) we have found no antigenic relationship of SHF virus to flaviviruses, nor to members of the other currently recognized togavirus genera and to lactic dehydrogenase virus, a togavirus unclassified to genus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 01983-14 ID

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Chronic Viral Infections - Molecular Biology of Human JC Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Eugene O. Major	Special Expert	IDB, IRP, NINCDS
Allen Aksamit	Medical Staff Fellow	IDB, IRP, NINCDS
Andra Miller	Microbiologist	IDB, IRP, NINCDS
Rene Traub	Microbiologist	IDB, IRP, NINCDS
Dominick Vacante	Staff Fellow	IDB, IRP, NINCDS
Sidney Houff	Clinical Associate	IDB, IRP, NINCDS
William T. London	Veterinary Director	IDB, IRP, NINCDS
Joseph Bressler	Senior Staff Fellow	SNB, IRP, NINCDS

COOPERATING UNITS (if any)
Surgical Neurology Branch, NINCDS
Microbiological Associates, Bethesda, Maryland

LAB/BRANCH
Infectious Diseases Branch

SECTION
Unit on Molecular Virology and Genetics

INSTITUTE AND LOCATION
NINCDS, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	2.5	2.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our studies continue on the molecular pathology of JC virus and human glial cells in culture, and more recently, with human brain tissue sections from infected patients with progressive multifocal leukoencephalopathy (PML). Experiments have focused at the intracellular level describing the nature of viral persistence in the CNS, pathogenesis of PML as a demyelinating disease, and the mechanisms of genetic regulation of the viral and cellular genes involved. Our results include solving the biggest problems that working with JCV has presented, namely the host and tissue restriction for growth to primary human fetal glial cells in culture. We have established an immortalized line of human fetal astroglial cells using a mutant transforming gene of SV40. These cells are able to produce infectious JCV and have allowed us to examine the kinetics of JCV DNA replication and T protein synthesis. We have also found that the SV40 T protein made in human glial cells complexes with a glial cell protein, p53, and stabilizes this protein in the cell. The JCV T protein does not appear to complex with the p53 protein, perhaps due to conformational differences between the T proteins of SV40 and JCV. Additional evidence for such differences came from our experiments suggesting alkylation of cysteine residues of the JCV T protein was necessary in order to resolve the immunoprecipitated T protein using denaturing gel electrophoresis. This was not necessary for either the T proteins of SV40 or the related human BK virus. Studies of paraffin embedded or frozen brain tissue from PML patients showed the presence of JCV DNA using in situ hybridization and correlated with the detection of viral capsid antigen using immunocytochemical tests. These studies also revealed that oligodendroglial cells are the main target for JCV gene expression but that bizarre astrocytes present in PML plaques showed JCV DNA and capsid antigen. This latter finding questions the role JC virus may have in the pathogenesis of malignant gliomas in the general population that has been suggested by others.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-NS-02602-02-ID

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Border Disease Virus: Structure, Replication and Pathogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Barbara J. Potts	Staff Fellow	IDB, IRP, NINCDS
Other:	Gregory A. Elder	Medical Staff Fellow	IDB, IRP, NINCDS

COOPERATING UNITS (if any)
University of California Davis, Dept. Path., School of Vet. Med., Davis, CA; United States Diagnostic Services, USDA, Ames, Iowa; Microbiological Associates Inc., Bethesda, Maryland

LAB/BRANCH
Infectious Diseases Branch

SECTION
Immunochemistry and Clinical Investigations Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Border Disease (BD) of sheep is a viral induced dysmyelinating disorder caused by a virus in the genus Pestivirus and the family Togaviridae. BD virus, when acquired congenitally, causes abortions or multiple malformations of the CNS, the skeletal and immune systems and of the integument. In the CNS, the affected lambs have cerebellar tremors, are microencephalic and the only histopathological lesions seen in this disease are a reduction in myelin and a glial proliferation. This research program focuses on identifying the mechanisms of microencephaly and myelin reduction in these lambs with congenital BD. Experimental methods involve pathogenesis studies using immunoglobulins as anatomical and biochemical probes of the nervous system as well as biochemical methods to characterize and isolate BD viral polypeptides. In our previous studies we demonstrated that persistent BD virus could only be acquired by a congenital infection, suggesting a viral trophism for precurser cells in the CNS and the lymphoreticular system. To test this hypothesis, primary cell cutures have been developed from fetal and adult ovine CNS tissues and from peripheral white blood mononuclear cells. Control sheep and a lamb with congenital BD were studied. Using dual immunocytochemical labeling studies, we found that all cell types in tissue culture from fetal and adult ovine tissues were susceptible to BD viral infection. These findings suggest that the restriction of BD viral replication in the animal may not be due solely to cell trophism for precurser cells. In preparation for electron microscopic localization of BD viral proteins, we have developed the techniques to visualize the BD virion, which is spherical and 70 nm in diameter. We have also been able to prepare infected cells where cellular morphology and BD viral antigenicity are maintained. These data suggest that the BD viral proteins are intracellular and associated primarily with membranes. BD virus in sheep and in tissue culture is an important tool for investigating the mechanisms of myelinogenesis and viral persistence.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	**PROJECT NUMBER** Z01 NS 02531-04 ID

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies in Neuromuscular and CNS Diseases and Their Experimental Models

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Marinos C. Dalakas, Senior Staff Fellow, IDB, IRP, NINCDS

John L. Sever	Chief	IDB, IRP, NINCDS
David L. Madden	Veterinary Director	IDB, IRP, NINCDS
Maneth Gravell	Research Microbiologist	IDB, IRP, NINCDS
William T. London	Veterinary Director	IDB, IRP, NINCDS

COOPERATING UNITS (if any)
George Washington University Med. Ctr., Johns Hopkins University Medical School

LAB/BRANCH
Infectious Diseases Branch

SECTION
Immunochemistry and Clinical Investigations

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1	.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Enzyme histochemistry in muscle and nerve biopsies is carried out for diagnostic purposes in patients with several neuromuscular disorders. Immunocytochemical studies were conducted using specific antibodies to thymic peptides to investigate changes in the distribution of epithelial cells in the thymus of patients with myasthenia gravis. Using the cytofluorograph, specific subsets of lymphocytes that carry thymic markers (thymosin alpha$_1$, alpha$_7$, beta$_4$) were defined. The interaction between cells of the lymphoid and central nervous system was investigated searching for common antigenic markers on their cell surface. Thymosin beta$_4$, an immunomodulating polypeptide, was found to be a common antigen shared by macrophages, dendritic lymphoid cells and oligodendrocytes. The IgM of certain patients with paraproteinemic polyneuropathies has been identified as a specific antibody to myelin associated glycoprotein or glycolipids; nerve biopsies from these patients are studied by electron microscopy and immunocytochemically. The nature of amyloid protein in patients with "sporadic" amyloid polyneuropathy was identified using specific antibodies to amyloid proteins immunocytochemically on tissue sections and biochemically on the extracted amyloid. Immune cellular markers were investigated during the evolution of EAN and EAE induced in rhesus monkeys and therapies were attempted using some novel immunomodulating agents. The mechanism of inflammatory myopathy in monkeys with immunodeficiency (Simian AIDS) due to a retrovirus D, was further studied. Antibodies to the retrovirus immunoreacted with the inflammatory cells invading the muscle fibers; the retrovirus was capable of infecting myoblasts in tissue culture without exerting a cytopathic effect in the muscle. The effect of aging on the neuromuscular system of monkeys from age 5 to 25 is being investigated with a detailed morphological and morphometrical analysis of their muscle and nerve biopsies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-NS-00972-14-ID

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Animal Models for CNS Infections in Normal and Immunocompromised Hosts

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	William T. London	Veterinary Director	IDB, IRP, NINCDS
Others:	Maneth Gravell	Research Microbiologist	IBD, IRP, NINCDS
	Val G. Hemming	Associate Professor	USUHS
	John L. Sever	Chief	IDB, IRP, NINCDS
	Sidney A. Houff	Neurologist	IDB,IRP, NINCDS
	Marinos C. Dalakas	Senior Staff Fellow	IDB,IRP, NINCDS
	A. Julio Martinez	Prof. Neuropathology	U. PITT SCH MED
	Jere M. Phillips	Dir, Animal Medicine	MELOY LAB.
	Blanche L. Curfman	Biologist	IDB,IRP, NINCDS
	Robert L. Brown	Biological Lab Technician	IDB,IRP, NINCDS

COOPERATING UNITS (if any)
Uniformed Services University of the Health Sciences, Bethesda, Maryland; University of Pittsburgh Presbyterian Hospital, Department of Neuropathology, Pittsburgh, Pennsylvania; Meloy Laboratories, Inc., Springfield, Virginia

LAB/BRANCH
Infectious Diseases Branch

SECTION
Experimental Pathology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.10	.80	2.30

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Group B streptococci (GBS) are a major cause of neonatal sepsis and meningitis. Our studies in the rhesus monkey model suggest protection from GBS infection was not significantly associated with maternal antibody titer, prior immunization history or dose of GBS given. Maternal IgG may be insufficient to protect the unborn fetus should a GBS amniotic infection occur during partuition.

Neuromuscular changes in rhesus monkeys of various ages:

In our studies of polymyositis in rhesus monkeys we have found several neuromuscular changes not previously well described for this species. A study designed to observe possible neuromuscular changes associated with ageing of these animals was done. Preliminary findings show that changes of denervation start as early as age 10 and progress slowly there after.

Simian Varicella (SV):

A recent isolate of SV has been studied in several species of mokeys. Rhesus monkeys appear to be the best experimental host. SV virus produces a mild disseminated chicken pox-like infection. This model offers many advantages for study of human latency and diseases associated with Varicella Zoster.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-NS-01986-14-ID

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Inoculation of Animals with Tissue Culture Grown Materials from Patients with Chronic Neurological Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	William T. London	Veterinary Director	IDB, IRP, NINCDS
Others:	Marinos C. Dalakas	Senior Staff Fellow	IDB, IRP, NINCDS
	John L. Sever	Chief	IDB, IRP, NINCDS
	Blanche L. Curfman	Biologist	IDB, IRP, NINCDS
	Robert L. Brown	Biological Lab Technician	IDB, IRP, NINCDS

COOPERATING UNITS (if any)
Meloy Laboratories, Springfield, Virginia

LAB/BRANCH
Infectious Diseases Branch

SECTION
Experimental Pathology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated and the remaining aspects of it transferred to Project Number Z01-NS-00972-14-ID.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-NS-02136-11-ID

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Control of Acute Infectious Diseases in Experimental Animals Using Biologicals and Chemotherapeutic Agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	William T. London	Veterinary Director	IDB, IRP, NINCDS
Others:	Maneth Gravell	Research Microbiologist	IDB, IRP, NINCDS
	Marinos C. Dalakas	Senior Staff Fellow	IDB, IRP, NINCDS
	John L. Sever	Medical Director, Chief	IDB, IRP, NINCDS
	Blanche L. Curfman	Biologist	IDB, IRP, NINCDS
	Robert L. Brown	Biological Lab Technician	IDB, IRP, NINCDS

COOPERATING UNITS (if any)
Meloy Laboratories, Springfield, Virginia

LAB/BRANCH
Infectious Diseases Branch

SECTION
Experimental Pathology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated and the remaining aspects of it transferred to Project Number Z01-NS-00972-14-ID.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02271-09-ID

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Papovaviruses in Nonhuman Primates

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	William T. London	Veterinary Director	IDB, IRP, NINCDS
Other:	Sidney A. Houff	Research Associate	IDB, IRP, NINCDS
	Eugene Major	Special Expert	IDB, IRP, NINCDS
	John L. Sever	Chief	IDB, IRP, NINCDS
	Blanche L. Curfman	Biologist	IDB, IRP, NINCDS
	Robert L. Brown	Biological Lab. Technician	IDB, IRP, NINCDS

COOPERATING UNITS (if any)

University of Wisconsin Medical School, Departments of Medical Microbiology and Pathology, Madison, Wisconsin

Meloy Laboratories, Inc., Springfield, Virginia

LAB/BRANCH

Infectious Diseases Branch

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated and the remaining aspects of it transferred to Project Number Z01-NS-01983-14-ID.

TAB 17 -- MEDICAL NEUROLOGY BRANCH -- (MNB)

ANNUAL REPORT

October 1, 1984 through September 30, 1985

Medical Neurology Branch, IRP

National Institute of Neurological and Communicative Disorders and Stroke

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ANNUAL REPORT
October 1, 1984 through September 30, 1985

Medical Neurology Branch, IRP
National Institute of Neurological and Communicative Disorders and Stroke

Chief, Roger J. Porter, M.D.

The Medical Neurology Branch was officially re-established within the Intramural Research Program of NINCDS on June 27, 1984. On January 21, 1985, the Clinical Neuropsychology Section was transferred from the Clinical Neurosciences Branch to the Medical Neurology Branch. The Branch conducts research on human epilepsy, including new approaches to diagnosis and treatment, investigates basic questions related to normal and abnormal neuronal excitability, performs studies on human motor control and speech, conducts research on Alzheimer disease and related disorders including autonomic dysfunction, and investigates cognitive and emotional processes in man.

The Branch is divided into five approved sections. Roger J. Porter, M.D., is Chief of the Clinical Epilepsy Section, Mark Hallett, M.D. is Chief of the Human Motor Control Section, Ronald J. Polinsky, M.D. is Chief of the Clinical Neuropharmacology Section, and Paul Fedio, Jr., Ph.D. is Chief of the Clinical Neuropsychology Section. The position of Chief, Neuronal Excitability Section is vacant.

Clinical Epilepsy Section

The Clinical Epilepsy Section is undertaking a series of studies using techniques of intensive monitoring of patients with intractable seizures in order to improve clinical control in patients with refractory seizure problems, and to aid in the diagnosis of patients with disorders of unknown type such as psychogenic seizures. Emphasis is being placed on positron emission tomography (PET) as a technique to investigate basic mechanisms of cerebral metabolism in epilepsy and to assist in the clinical evaluation of patients with severe partial seizures. Ultrastructural and biochemical investigations of epileptic tissue removed at surgery will be correlated with metabolic findings. Studies have begun with magnetic resonance imaging (MRI), which has potential for more precise localization of epileptogenic lesions, as well as for elucidation of the anatomical substrates of altered physiologic patterns revealed by PET.

Patients with severe uncontrolled seizures are admitted to the Clinical Center according to the following criteria: 1) patients with complex partial sizures, especially those who may be candidates for PET scan evaluation and surgical therapy; 2) patients with absence seizures or atonic/myoclonic for studies of cerebral metabolism including the effect of antiepileptic drugs. After seizure frequency and type is characterized by intensive monitoring techniques, the patients are placed in an appropriate research protocol. After the research protocol is completed, each patient's therapeutic regimen is adjusted to obtain optimal seizure control.

PET is a technique using the intravenous injection of a radioactive isotope to determine regional rates of cerebral metabolism. The Clinical Epilepsy Section has been using (^{18}F)-fluorodeoxyglucose (FDG) to measure the regional cerebral use of glucose over a 30-minute period after the injection of isotope. Ongoing studies include (1) patterns of cerebral metabolism in patients with partial, generalized, and atonic/myoclonic seizures; (2) the effect of antiepileptic drugs on cerebral metabolism.

The role of MRI scanning in the seizure disorders is also being investigated. MRI shows more detailed anatomic images than CT, and may detect subtle changes in cerebral density resulting from small gliotic regions which may be epileptogenic.

A project has been initiated to use sphenoidal, and in some cases subdural electrodes, in the evaluation of potential surgical candidates, coupled with long-term video-EEG recording techniques. These techniques allow the acquisition of EEG data not available via surface recordings. This data is correlated with PET and MRI to obtain the best possible presurgical localization of epileptic foci. A prospective evaluation of the relative value of invasive (subdural) and noninvasive methods of presurgical evaluation has begun.

Magnetoencephalography (MEG) is a new approach to the problem of localizing abnormal cerebral potentials which may represent an epileptic focus. Initial studies suggest that MEG may provide more precise three-dimensional information than EEG, allowing detection and localization of epileptic foci in the depths of the brain, without the need for invasive procedures.

Clinical Pharmacology of Antiepileptic Drugs

Pharmacologic studies in epilepsy continue to concentrate on studies of drug interactions and of new antiepileptic drugs.

Patients with uncontrolled seizures, especially complex partial seizures or absence seizures, are accepted for study. Such patients usually have a detailed seizure calendar available prior to entering the study; they enter a week-long period of baseline determination of seizure frequency and blood levels of antiepileptic drugs while in the hospital. Each pharmacologic protocol varies, but all require modification of the antiepileptic regimen and addition of the medication under study. This may be done in single dose or chronic administration studies depending upon the particular protocol in question. Plasma levels are often drawn daily, and on occasion, much more frequently for specific studies. Following the completion of the pharmacologic protocol, the patient is placed on a regimen which is best suited for the seizure type which has been identified by videotape/telemetered EEG analysis. This regimen is stabilized prior to discharge of the patient.

The use of phenytoin is complicated by its saturable metabolism and nonlinear kinetics at steady-state concentrations. Because the excretion rate is not proportional to dose, when the dose is close to the maximum rate of metabolism, the plasma levels of the drug may rise to unexpectedly high levels after small dose increases. We evaluated the course of plasma phenytoin levels after 13 dosage changes in ten patients on long-term phenytoin treatment and described a "pseudo-steady-state" phenomenon in which the plasma phenytoin levels were apparently stable but subsequently changed before a final steady state level was reached. The changes were of clinical relevance in some patients. Although Michaelis-Menten kinetics may predict the pseudo-steady-state phenomenon at high plasma phenytoin levels, a multi-compartment kinetic model with prolonged distribution equilibrium is more likely to account for our observations.

HUMAN MOTOR CONTROL SECTION

The mission of the Section is to understand normal principles of motor control in man and the pathophysiology of motor disorders including both deranged voluntary movement and involuntary movement. The Section is composed of a Speech Pathology Unit and a Motor Disorders Unit.

Speech Pathology Unit

In the Speech Pathology Unit neurophysiological studies of speech and phonatory control have been initiated to provide direct examination of speech motor control in various neurological diseases. The research has become more focused on specific aspects of speech timing and phonatory control. The research goal continues to be to determine the neurological organization of speech production and phonatory control through the study of the breakdown of these functions in neurological disorders. The objectives of the three current projects are:

1. To determine the neurophysiological bases of phonatory function in normal and disordered voice.

2. To determine which aspects of speech timing are independently controlled in the nervous system from studying speech disorders in different neurological diseases.

3. To determine the organization and inter-relationships between speech production, speech perception and language in the nervous system from the study of these functions in various neurological diseases.

Phonatory Function: Two studies, determining the validity of methods used widely for assessing phonation, were completed this year. First, the validity and reliability of perceptual ratings for assessing phonation in patients with vocal fold nodules and polyps were determined. A 13-dimension perceptual rating system, modelled after perceptual rating systems currently used clinically, required eight hours of training to achieve inter-rater reliability in four listeners. The trained listeners then rated vowel phonations of patients and controls blind. Inter-and intra-judge reliability were adequate for only five dimensions. However, validity was demonstrated with 100 percent correct assignment to group when a discriminant function was computed employing all dimensions.

Acoustic measures have the advantage over perceptual ratings of being both objective and reliable. Measurement of phonatory frequency and amplitude perturbation (jitter and shimmer) is used by speech scientists for assessing phonation. Last year, we completed a study demonstrating that jitter was not valid for detecting patients with vocal fold disorders.

This experiment was repeated employing both jitter and shimmer. Since these measure different acoustic attributes, they were expected to be sensitive to different vocal fold abnormalities. However, the combined accuracy of jitter and shimmer was only 76 percent correct in assigning normal and pathological cases. Further, patients with different types of laryngeal pathology (nodules/polyps, edema, carcinoma and unilateral paralysis) were not selectively impaired on either of these measures. Thus, jitter and shimmer were not as sensitive to pathology as perceptual ratings and were not specifically affected by particular laryngeal pathologies.

We have begun the development of other acoustic measures which might reflect particular changes in laryngeal physiology affecting phonation. Based on the engineering concept of "slew", we extracted slow linear changes in cycle length and cycle amplitude for characterizing phonatory tremor. The rate and slope of frequency and amplitude oscillations were measured in cases with fiberoptically observable vocal fold tremors for comparison with other involuntary movement disorders affecting phonation. The slope of cycle length change and the variation in linear change in cycle length and cycle amplitude were increased relative to normal only in those with benign essential tremor of the vocal folds. Thus, intrinsic laryngeal muscle tremor could be characterized and differentiated from other disorders by these measures.

The normal rate of phonatory tremor was between 4 and 11 Hz with a mean of 6 Hz, which is slower than physiological tremor found in limb muscles. Further, tremor rate did not differ between the normals and patients with benign essential tremor or other involuntary movement disorders. Thus, phonatory tremor both in normalcy and pathology seems to differ from tremor in other parts of the body on other tasks. Neurophysiological studies are planned to examine the characteristics of muscle activity in the larynx in normalcy and pathology.

We have continued our studies of spasmodic dysphonia aimed at determining whether this is as an upper motor neuron disorder or a more peripheral neuropathology. The coordination and prephonatory posturing of the larynx with respiratory movements of the rib cage and abdomen were studied during a simple reaction time task. Since most spasmodic dysphonic patients do not have interruptions during whisper but do on phonation, both responses were studied. In comparison with normals, the patients had movement abnormalities and discoordination on phonation, but not on whisper. These patients' difficulties were task specific, not commensurate with a peripheral problem and suggestive of a focal dystonia, which is central in origin.

Speech Timing: Studies comparing speech timing breakdown in different neurological disorders have continued. A patient group with a speech dysprosody (acquired stuttering) following penetrating head injury was compared with normal controls on measures of speech initiation time, speech execution time, alterations in execution time, speech rate and alterations in rate. Brain lesions in this group involved the white matter tracts and the basal ganglia on either the right or left sides. The dysprosodic group was impaired in speech initiation and syllable repetition rate but not in execution time or alterations in execution time. In a previous study, patients with Huntington's and Parkinson's disease were impaired in execution time control and rate control respectively, but neither group was impaired in speech initiation time. Since white matter was affected only in the dysprosodic group, normal speech initiation time may be dependent upon white matter function.

Patients with Parkinson's disease are often unintelligible in their connected speech. It is not known whether this is a result of their rate control problem or speech articulation errors. Three syllables differing only in place of articulation were produced by patients and controls during a simple reaction time task. The syllables were than presented to listeners in a forced choice task to determine which syllables were unintelligible. Even for unsophisticated listeners, almost all syllables were correctly identified, although acoustic measures of location and rate of change of the first and second formants were affected in many patient productions. This suggests that Parkinson patients do not have significant articulatory errors in isolated speech syllables, but rather that intelligibility becomes reduced as a result of their rate control difficulties in connected speech. This same experiment will be repeated in connected speech to determine the effects of speech rate on patients' speech articulation accuracy and intelligibility.

Organization of Speech and Language: In an effort to determine the separability of speech perception from speech production and language skills, a group of head injured patients without speech production or language difficulties were selected for speech perception testing. Half of the group had speech perception deficits for voicing and place discrimination. Analysis of CT scans demonstrated that the lesion locations most clearly associated with the speech discrimination deficits were upper levels of the white matter subadjacent to cortical regions in either hemisphere. This demonstrated that when speech perception deficits occur in isolation, they can result from subcortical deficits rather than damage to the left temporoparietal cortex.

Motors Disorders Unit

The Motor Disorders Unit has grown during this period, achieving fully operational size in July 1985. No projects have been completed, but a number of projects are in progress. The major objectives are:

1. Physiological characterization and understanding of certain involuntary movements including myoclonus, tremor and unusual disorders.

2. Trial of isoniazid (INH) for amelioration of action tremors of various etiologies.

3. Physiological characterization of difficulties with balance experienced by patients with Parkinson's disease and cerebellar disorders.

4. Clinical and physiological characterization of hemiplegia resulting from brain injury, most typically stroke.

Involuntary movements: Myoclonus and a number of other rapid involuntary movements have been difficult to classify clinically. This trouble leads to inappropriate therapy and confusion for the patients. Consolidation of patient material obtained outside of NIH with continuing analysis of new cases at NIH has led to new classifications and pathophysiological insights. Conclusions have been drawn about myoclonus of epileptic nature, adult onset tic and startle.

Tremors are also difficult to classify. In studying the physiological aspects of these disorders, we have identified a new descriptor of cerebellar postural tremor which may help distinguish it from other postural action tremors. The amplitude of cerebellar postural tremor appears to depend on the precise posture of the limbs, and, in particular, is highest when the arms are near the body and the hands are pointing toward each other. The physiological implications of this are being explored. We have also studied several patients with orthostatic tremor, a curious, newly described disorder of tremor of the legs only when standing. Our preliminary studies suggest that this is a disorder of the balance mechanism.

We plan to continue these studies and add PET scanning as a physiological tool to our battery of clinical neurophysiological methods to help identify abnormal areas in the brains of patients with involuntary movements.

Trial of Isoniazid for Action Tremors: Previous studies have shown utility of isoniazid for ameliorating cerebellar postural tremor in patients with multiple sclerosis. Current studies are aimed at identifying whether patients with action tremors of other types are also benefitted. A double-blind, placebo-controlled, cross-over trial is in progress.

Balance Disorders: A laboratory is being set up for analysis of balance. This will enable simultaneous study of body angles, foot-floor forces and multiple EMGs. We hope to identify normal mechanisms of balance and how these are deranged in patients.

Hemiplegia: The study of motor control in hemiplegia is being set up as the major project of the Unit. Patients with discrete brain lesions will be studied; patients with strokes will be the main group, and many patients will be followed serially from onset of the disorder to recovery. We hope to discover pathophysiological mechanisms including those mechanisms underlying recovery. Methods of study will include: (1) quantitative clinical battery, (2) evoked potentials, (3) pre-motor potentials, (4) stretch reflexes, (5) EMG analysis of voluntary movements, and (6) PET scanning during voluntary movement.

CLINICAL NEUROPHARMACOLOGY SECTION

The Clinical Neuropharmacology Section continues to develop clinical, physiological, biochemical and pharmacological methods for assessment of autonomic nervous system function in man. Since norepinephrine is the neurotransmitter released by most post-ganglionic sympathetic nerve endings and is also an important central nervous system neurotransmitter, these investigations have focused primarily on the noradrenergic system. High performance liquid chromatography, liquid scintillation spectrometry, and mass spectroscopy are used to measure neurotransmitter and metabolite levels in plasma, urine, and cerebrospinal fluid under basal conditions and after a variety of stimuli have been applied to elicit a sympathetic response. Two groups of patients with chronic autonomic failure have been studied in order to elucidate the biochemical and pharmacological differences between central and peripheral autonomic dysfunction. Patients with idiopathic orthostatic hypotension (IOH) have pure or isolated autonomic failure in contrast to patients with multiple system atrophy (MSA) in whom the autonomic dysfunction is attended by a central nervous system disorder. Investigation of patients with lesion(s) of the autonomic nervous system has also provided an opportunity to determine the interaction between the autonomic nervous system and other hormonal/peptide systems.

Our previous studies of plasma catecholamines during insulin-induced hypoglycemia have shown that most patients with either MSA or IOH have deficient epinephrine and norepinephrine responses. These results were confirmed in an investigation of the relationships among beta-endorphin, enkephalin, ACTH, and catecholamine responses to hypoglycemia. In normal subjects, there was a striking rise in epinephrine, beta-endorphin, and ACTH following the nadir of hypoglycemia. In MSA patients, neither beta-endorphin or ACTH levels increased significantly, whereas in IOH patients these responses were normal. There was no correlation between the degree of adrenergic insufficiency and the beta-endorphin and ACTH responses. These results suggest that the central nervous system lesion(s) in MSA interfere with release of beta-endorphin and ACTH in response to hypoglycemia. The normal beta-endorphin and ACTH responses in IOH are consistent with involvement limited to the peripheral sympathetic nervous system. The strong correlation between beta-endorphin and ACTH responses is consistent with the common origin of these peptides. The dissociation between the catecholamines and peptide responses suggests that peripheral adrenergic activity is not essential for beta-endorphin and ACTH release in man. Further studies are planned to investigate whether the release of other pituitary and/or hypothalamic peptides are altered in patients with autonomic dysfunction.

Insulin administration results in hypotension as well as hypoglycemia in patients with autonomic neuropathy. In our studies of insulin-induced hypoglycemia a precipitous, sustained drop in mean blood presure occurred in IOH patients within 10 minutes following insulin administration. The drop in blood pressure preceded the increase in epinephrine levels. Propranolol did not alter the initial hypotensive effects of insulin but there was a significant improvement by 45 minutes after insulin administration. Thus, the initial drop in blood pressure does not appear to be mediated through excess vascular beta-adrenergic receptor stimulation in muscle. However, the later improvement in blood pressure observed following propranolol is likely the result of enhanced vasoconstrictor (pressor) activity of epinephrine occurring as a result of beta-adrenergic blockade. Preliminary analysis of the relationships among blood pressure, norepinephrine, and beta-endorphin responses suggests that beta-endorphin released without an adequate compensatory increase in norepinephrine may play a role in the genesis of insulin-induced hypotension. In order to further elucidate the mechanism of this phenomenon, additional insulin tolerance tests must be performed. The effects of hypoglycemia will be separated from the other consequences of insulin administration by using the glucose-clamp technique. Naloxone will be used to assess the role of endorphins in causing insulin-induced hypotension.

A number of additional studies involving patients with progressive autonomic failure, narcolepsy, and other neurological disorders are currently in progress:

1) Magnetic resonance imaging (MRI) in MSA patients has revealed a decrease in signal intensity in the putamen. This finding is consistent with the known neuropathology of Shy-Drager syndrome. A similar abnormality has been observed in one IOH patient; the meaning of this finding is unclear. However, demonstration of a central nervous system lesion(s) in IOH would provide evidence in support of the suggestion that IOH represents a form fruste of Parkinson's disease. Our analysis of cerebrospinal fluid monoamine metabolites in IOH patients reveals a slight but significant reduction in the levels of homovanillic acid, the major metabolite of dopamine in man. Further studies of the relationship between plasma and CSF levels of neurotransmitters and their metabolites are in progress; these investigations may help to explain the reduction in CSF homovanillic acid observed in IOH.

2) Although the studies of norepinephrine plasma disappearance kinetics and stereospecific labelling of urinary norepinephrine metabolites have been completed, the specific activity of red blood cell norepinephrine will be measured in samples which have been collected before and during infusion of a mixture containing radiolabelled levo and dextro isomers of norepinephrine and isoproterenol. The red blood cell may serve as a model for studying neuronal uptake.

3) Cardiovascular and catecholamine responses to intravenously administered acetylcholine are being studied in patients with MSA and IOH. The purpose of these studies is to look for evidence of ganglionic supersensitivity which may differ according to the site of the autonomic nervous system lesion.

4) A pilot study of norepinephrine subsensitivity has been completed. Analysis of the plasma norepinephrine levels is in progress. Investigation of this phenomenon is critical for further development of an implantable sympathetic nerve prosthesis that could be used for chronic management of orthostatic hypotension.

5) Biochemical and pharmacological investigation of autonomic nervous system function in several members of a family with a previously unrecognized form of leukodystrophy has been completed. Clinically, these patients have orthostatic hypotension as well as other signs of autonomic dysfunction. Preliminary analysis of the results reveals low supine plasma norepinephrine levels and very little increase in epinephrine during insulin-induced hypoglycemia. Thus, it appears that this disorder may be attended by peripheral sympathetic nervous system dysfunction that includes deficient adrenal medullary responses.

6) Studies of autonomic function in narcolepsy and aging have continued. Additional subjects must participate before meaningful results can be obtained.

7) Data collection has been completed for the clinical and family studies involving 55 subjects with progressive autonomic failure. There does not appear to be an hereditary pattern of either MSA or IOH. A more complete analysis of many aspects of clinical expression, associated disorders, and other factors is in progress.

The Clinical Neuropharmacology Section has continued the study of familial Alzheimer's disease as a major priority within the scope of its research efforts. This project has evolved from an initial investigation of a large family with histologically confirmed Alzheimer's disease from New Brunswick, Canada. Alzheimer's disease is a major medical and social problem since it is the most common cause of irreversible, chronic dementia. The studies of Alzheimer's disease are significantly limited by both accuracy and timing of diagnosis. Unfortunately, the diagnosis of Alzheimer's disease must be left to the neuropathologist. This complicates clinical research studies since more than 20% of clinically diagnosed cases do not have Alzheimer's disease at autopsy. Although Alzheimer's disease may be inherited in less than 10-20% of all cases, the main justification for studying familial cases lies in the accuracy of diagnosis which may be inferred through post-mortem examination of other affected family members.

There are two major aims of our studies. One is to investigate genetic linkage in order to define the chromosomal abnormality in familial Alzheimer's disease. This may ultimately allow identification of the gene product which will elucidate the underlying pathophysiology and hopefully stimulate more rational therapeutic approaches. The second aim is to define the clinical and biochemical progression of the disease through a longitudinal investigation of affected and at-risk subjects. Neuropathological and neurochemical studies of post-mortem specimens from these families will also be conducted. These studies will hopefully provide clues for earlier and more accurate diagnosis that will facilitate research on sporadic cases.

Clinical and family studies are in progress. A preliminary analysis of an Alzheimer's disease study involving 26 pairs of twins has revealed an heterogeneous pattern of concordance in both identical and non-identical twin pairs. This would suggest that factors other than heredity are important for the development of Alzheimer's disease. Ten families with histological confirmation of Alzheimer's disease are also under investigation. The inheritance pattern in these families is consistent with an autosomal dominant pattern. A study of the clinical expression and neuropathological correlates of the disease in these families is in progress.

The longitudinal investigation of affected and at-risk subjects from families with apparently dominant inheritance of Alzheimer's disease has continued. A number of studies are conducted with these subjects including: EEG, evoked potentials, CAT scans, detailed neuropsychological testing, and PET scanning. Biochemical and neuropharmacological assessments of neurotransmitter metabolism and peptides are also being performed. The number of subjects is currently not sufficient to permit a meaningful analysis of the data.

We have also completed several field expeditions to perform clinical evaluations and obtain skin biopsies and blood samples on selected members of these large families. Skin fibroblast and peripheral blood lymphoblast cultures are being established. These cultures will serve as a renewable source of DNA and cell lines which can be used for genetic linkage, viability, and biochemical studies. Collaborative arrangements have been made with several laboratories to investigate genetic markers including DNA restriction fragment length polymorphisms. The initial genetic marker research efforts have focused on chromosome 21. This decision was based on the observations in post-mortem brain specimens from Down's syndrome (most often caused by Trisomy 21). Brains from these patients show neuropathological abnormalities which resemble the findings in Alzheimer's disease. No definite linkage has been found in the preliminary analysis of the results obtained on specimens from members of the large Canadian pedigree. However, the data do not exclude chromosome 21, and it may be necessary to test additional probes. Collaborative arrangements are in progress to test specific single probes of interest including those for somatostatin and choline acetyltransferase.

The post X-ray survival of lymphoblastoid lines from patients with Alzheimer's disease was studied using the trypan-blue dye-exclusion test. Cells from patients with Alzheimer's disease, Parkinson's disease, and Down's syndrome had mean viability ratios less than the control group. This in vitro hypersensitivity to X-rays is likely the result of unrepaired DNA damage. Since these patients had the sporadic form of the disease, the defect causing the abnormality in DNA repair must have occurred at a developmental stage which did not affect the reproductive cells. Additional viability studies are planned using the cells from familial Alzheimer's disease patients.

CLINICAL NEUROPSYCHOLOGY SECTION

Cognitive and emotional activities in man are dependent upon the integrity of the limbic system, and therefore, insult to this brain region affords neuroscientists an unique opportunity to examine and to better understand the neural basis of human behavior. In a series of neuropsychological studies, perceptual mechanisms were evaluated in epileptic patients following a unilateral left or right temporal lobectomy. Using rapid delivery, tachistoscopic projection, right temporal patients required a longer exposure duration to detect the presence of a stimulus, but not to discriminate two versus single flashes. Left temporal patients, in contrast, exhibited the reverse pattern. Tachistoscopic delivery of chimeric random shapes and words revealed that recognition of the left and right sides of the stimuli varied independently. The right temporal patients had a significantly lower accuracy for the left as compared to the right side of the stimuli. In contrast, the left temporal patients and control subjects showed a lower accuracy for the right hemi-field. These data suggest that right hemisphere mechanisms are optimally suited for summation of sensory input over time to yield heightened perceptual sensitivity, but at the expense of fine temporal resolution. Left temporal systems are better organized and suited to deal with fine temporal acuity, but at the expense of overall perceptual sensitivity. Additional studies of the temporal lobectomy patients also indicated that spatial location is less dependent on the integrity of the anterior and medial temporal lobes.

There appears to be an asymmetry in the organization and expression of emotionality by the left and right limbic systems. For example, left temporal lobectomy patients expressed neutral ratings about scenic material that were ranked as pleasant or horrific by normal subjects. Patients with left removal also applied inappropriate verbal descriptions for visually presented sequences of emotional behaviors. The same response bias held for these patients in judging photographs of faces displaying different emotional expressions. There was less disruption by a right temporal resection. Moreover, the left and right temporal patients were physiologically unresponsive while viewing affective material, as indexed by skin conductance indices. Unlike normal subjects, the temporal lobectomy patients were unable to take advantage of the emotional coloration of information in facilitating subsequent recall.

Relatedly, the left and right temporal patients evaluated their behavior differently on a behavioral inventory. In comparison with nonoperative epileptic subjects, most patients acknowledged an improvement in their behavior following unilateral temporal lobectomy. Nonetheless, within the context of this general improvement, specific personality styles persisted and were dependent on the side of removal: the left temporal lobectomy patients viewed themselves as ideative, reflective and non-emotional, but were overly harsh and self-critical in their ratings; in contrast, the right temporal patients regarded themselves as emotive, but in a more socially favorable light than their raters.

In an effort to assess the compensatory value of mnemonic strategies, temporal lobectomy patients were instructed in the use of different encoding cues to deal with postoperative memory difficulties. The study confirmed the facilitatory value of visual imagery by the neurosurgical patients, and matched groups of medical neurological patients and normal individuals. Abstract, low-imagery words were poorly recalled by the left temporal patients. In a separate study, subjects received instructions to either print a word or to sketch a picture of an object represented by a word, overtly (graphically) or covertly ('in mind's eye'); all groups remembered fewer printed than sketched words, and the left temporal group did less well in remembering sketched material. In another mnemonic paradigm utilizing phonetic, spatial, or praxic cues, all groups, particularly the left, did very poorly with phonetic encoding. In contrast, spatial and praxic mnemotechnics proved beneficial, moreso for the left temporal patients. These data confirm that left temporal mechanisms are indispensable to encode verbal information during initial learning. Modest compensation for memory defects following temporal lobectomy may be achieved with strategies that combine overt or covert imagery with praxic encoding.

Extending these behavioral changes with computer derived, electrophysiological indices (P300 events), procedures were developed to analyze neural components of cognitive or judgemental processes. Following unilateral temporal lobectomy, P300 amplitude was found to be inversely proportional of stimulus probability. With auditory stimuli, P300 activity was essentially identical for both left and right temporal patients. In patients with left temporal surgery, smaller P300s were observed, owing to a negative shift which emerged approximately 90 msec after stimulus onset.

For the visual modality, right temporal patients manifested smaller P300s than left temporal or normal subjects. There were no consistent hemispheric asymmetries which distinguished the left and right temporal patients, suggesting more than one neurogenerator of the P300 event, independent of lateral or mesial temporal structures. Processing of auditory and visual material is at least, to some extent, lateralized or hemispheric dependent.

P300 activity was also studied in normal children and patients with Turner's syndrome. Wave forms from some of the 18 and 20 year old female patients resembled the patterns of normal, however, much younger children or those entering the age of puberty. These results underscore the role of sex hormones in the development of neuropsychological processes.

The developmental course of the P300 with normal children revealed a striking change in frontal negative slow wave across the age spectrum. The amplitude and duration of this negative waveform decreased with increasing age and was inversely related to stimulus event probability. Within conditions involving time and judgement, the P300 became more broad and peaked with advancing age. The changes in frontal negative slow wave were consistent with data from other reports, suggesting a maturation of frontal negativity which continues over the entire lifespan, and parenthetically, is altered by presenile dementia.

Visual, spatial, and constructional abilities were also examined with neuropsychiatric patients, those with Alzheimer's (AD) or Huntington's (HD) disorder. However, the pattern of deficits was different; HD patients exhibited relatively greater impairment on tests of spatial judgement (egocentric in comparison with extrapersonal spatial tasks) whereas AD patients showed the reverse pattern. These findings, viewed in the context of studies of patients with frontal vs parietal lobe lesions, implicate degeneration of frontal striatal mechanisms in Huntington's disease, and the primary dysfunction in AD is associated with atrophy of cortical association regions.

The central theme of investigations with neuropsychiatric patients indicated that, at least during the early stages, patients with Alzheimer's disease may present with qualitatively different cognitive profiles, corresponding regions of neuropathology, and patterns of decline. As a result, questions concerning the status of cognition and memory in these patients can not be meaningfully or adequately addressed if they are treated as a homogeneous group.

Standardized and experimental verbal perceptuomotor tests were administered to 43 AD patients and revealed marked individual differences. A factor and a cluster analysis of the data identified several subgroups, verified by a discriminant analysis which correctly reclassified 42 of the 43 patients. Three qualitatively different groups were identified: one group was comprised of patients with relatively equal, verbal and visuospatial impairment, another group displayed severe semantic memory deficits concurrent with intact visuoconstructive skills, and a third group was characterized by greater impairment of constructional skills relative to their ability to access semantic knowledge. External validation of these clusters was obtained by analysis of retest performance after a one to two year interval; there were different patterns of decline, dependent on initial group membership. Moreover, the regional positron emission tomogrpahy data (^{18}F FDG) revealed symmetrical hypometabolism of the temporal and parietal cortex in globally impaired patients; relatively greater hypometabolism of the right temporal and parietal regions in patients with visuoconstructive deficits; and hypometabolism of the left temporal lobe in the semantic memory group.

Episodic Memory: As expected, this short-term memory function was generally impaired for all groups for both verbal material (recall of stories, paired-associates, recall and recognition of word lists) and nonverbal information (reproduction and recognition of complex figures). However, a material-specific deficit limited to verbal material was found in some patients from the semantic-memory group which later progressed to a global impairment by the time retest.

Semantic Memory: The groups differed with respect to word-finding ability and its relation to other cognitive and episodic memory deficits. Analysis of the fluency responses and naming errors suggested that, even in the most impaired patients, access to broad categorical knowledge may be preserved. Evidence in support of this possibility was obtained by demonstrating that these patients could sort pictorial objects into appropriate categories and answer questions about superordinate features (living or man-made?) and a specific category (food, animal, or tool?). However, errors occurred when required to answer yes/no questions probing knowledge of specific attributes (eg., it is used to cut things? for a picture of a saw).

Procedural Learning: Patients were presented with an apparatus consisting of a 10 x 10 matrix of metal disks which, when touched by a metal stylus, signaled either a correct (low tone) or incorrect (high tone) choice. Subjects were required to discover and learn a fixed route and to remember and apply simple rules (one step at a time, no diagonal moves, return to the previous position after an error). A double dissociation was found in that patients from the semantic-memory group were able to learn the maze at a normal rate, but made a large number of rule-breaking errors (rarely committed by normals); patients with severe constructional deficits were unable to learn the route, but honored the rules.

There was a study involving a single AD patient with an unusual constructional disorder and a global, episodic memory impairment, but relatively intact access to semantic memory and visual-recognition ability. While his reproduction of a complex geometric figure was severely impaired, this deficit was dissociated to a remarkable degree from his ability to draw complex and meaningful scenes. Thus, this patient's ability to tap a formerly acquired perceptual motor skill was dependent, at least in part, on the meaningfulness of the material. Therefore, even within the domain of a relatively circumscribed ability (copying visual material), both preserved and impaired functioning can be observed and related to the integrity of other cognitive systems (e.g., semantic memory).

In collaboration with NICHD investigators, eight symptomatic Long-Term survivors of acute lymphoblastic leukemia (ALL) who received CNS preventive therapy (cranial irradiation and intrathecal chemotherapy) were studied. On the basis of CAT scan findings, statistical relations were calculated between radiographic and behavioral abnormalities. In essence, patients with abnormal CAT scans showed impairment in attentional, memory and learning processes, the poorest performance being shown by those with evidence of calcification.

Cerebral dysfunctioning of frontal mechanisms has been implicated in obsessive-compulsive symptomatology. Guided by this hypothesis, a series of perceptual and memory tasks were developed and administered to preadolescent and adolescent obsessive-compulsive patients (n=26) and matched normal subjects (n=24). The neuropsychiatric patients consistently did poorly with spatial learning and memory tasks, and procedures requiring left-right directional judgements or imaginary self-rotation in space. Organizational defects and atypical strategies were commonly observed with the neuropsychiatric subjects. The obsessive-compulsive patients also showed elevated thresholds for visual material tachistoscopically projected to the left, right, and central fields, and a sharp ear asymmetry with dichotic recall. These data were interpreted in the context of an inhibition-disinhibition dyscontrol. Defective neural regulators may propel automatic-stereotypic expressions of ideative and ritualistic behaviors in obsessive-compulsive disorders.

NEURONAL EXCITABILITY SECTION

The question of what causes an epileptic seizure has been investigated from many different points of view. The anatomical, physiological and biochemical aspects of the epileptic focus in humans and animal models have been investigated in detail. The specific questions which will be asked and attempted to be answered by the Neuronal Excitability Section are as follows:

a) Is the resting membrane potential of the epileptic focus different from normal brain regions?

b) Are the calcium ion alterations involved in epileptic seizures?

c) Is a breakdown of the structural protein fodrin by the proteolytic involved in the spread of depolarization?

The procedures to answer these questions are as follows:

a) The membrane potentials will be evaluated using the membrane permeant lipophilic cation (^3H) tetraphenyl phosphonium borate. When the concentration of this cation inside and outside cells or synaptoneurosomes is applied to the Nernst equation a resting potential of -65 ± 8 mV is obtained. This resting potential closely parallels that obtained in electrophoretic experiments. Thus this procedure will be adequate for obtaining the answer to question a.

b) Abnormalities in calcium ion flux will be evaluated in synaptoneurosomes from the epileptic focus and normal brain using radioactive calcium.

c) Fodrin is a structural protein, which is important in the maintenance of the integrity of the membrane. Its degradation by the thiol protease calpain leads to abnormalities in cell membrane proteins such as receptors, and ion channels. The role of the protein in the epileptic focus will be evaluated by gel electrophoresis techniques.

An additional problem in epilepsy has been the occurrence of sudden death in patients, with no apparent anatomical reason. The Neuronal Excitability Laboratory will be involved in evaluating the blood and CSF of epileptic patients whose heart rates are being monitored, with respect to levels of neurotransmitters, neuropeptides, prostaglandins, and leukotrienes before and after seizure activity. A correlation between the chemical and EKG findings will be attempted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02318-08 MNB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Clinical Pharmacology of Antiepileptic Drugs

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Roger J. Porter, M.D. Neurologist, Chief, MNB, IRP, NINCDS and Head, CES, MNB, IRP, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Medical Neurology Branch, Intramural Research Program

SECTION
Clinical Epilepsy Section (CES)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Clinical Epilepsy Section continues to study the clinical pharmacology of old and new antiepileptic drugs. In a study of a relatively old drug, the pharmacokinetics of phenytoin were studied, especially its saturable metabolism; a new observation, the pseudo-steady-state phenomenon was described, which aids in an understanding of the difficulties that physicians often have in utilizing this drug for effective, nontoxic care of their patients. In this model, a dosage adjustment may initially lead to rapid fluctuations in plasma drug levels because of changes in various body compartments, followed by a flattening of the plasma drug level curve as tissue and blood concentrations equilibrate. Subsequent changes in plasma drug levels occur as a result of distribution into and out of poorly diffused tissue. In another study of phenytoin, the rationale for studying free, rather than total phenytoin levels was investigated. The importance of establishing the role of free phenytoin determinations was important because some authors have suggested that free phenytoin levels correlate more closely than total levels with seizure control. In our study, free phenytoin measurements were only marginally better predictors of either drug toxicity or drug efficacy, allowing us to conclude that routine monitoring of such free levels is not warranted. New studies are planned with a promising dicarbamate, W-554. This compound, which has activity against maximal electroshock in rodents, will be studied in patients with uncontrolled partial seizures; the study will be a randomized, double blind, placebo-controlled study performed in the Clinical Center, NIH. Pilot studies at other institutions suggest that the drug may be very effective. All pharmacologic evaluations of antiepileptic drugs are coupled with efficacy studies, carried out by intensive monitoring techniques, including videotape analysis of epileptic seizures with simultaneous telemetered EEG recordings and daily determinations of antiepileptic drug levels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02236-10 MNB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Diagnostic and Therapeutic Reevaluation of Patients with Intractable Epilepsy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Roger J. Porter, M.D. Neurologist, Chief, MNB, IRP, NINCDS and
Head, CES, MNB, IRP, NINCDS

COOPERATING UNITS (if any)
Office of Administrative Management;
Clinical Center, NIH
Office of the Clinical Director, NINCDS

LAB/BRANCH
Medical Neurology Branch, Intramural Research Program

SECTION
Clinical Epilepsy Section (CES)

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Clinical Epilepsy Section has been developing and testing new techniques to improve seizure control, medication tolerance, and rehabilitation in patients with severe epilepsy. Patients with uncontrolled seizures are admitted for a complete evaluation, including simultaneous video and telemetered EEG recording of seizures, daily determinations of antiepileptic drug serum concentrations, positron emission tomography (PET), magnetic resonance imaging (MRI), and magnetoencephalography (MEG). A specific seizure diagnosis is established allowing each patient to be assigned to an appropriate research protocol and therapy.

PET in patients with localized brain lesions has demonstrated focal hypometabolic cerebral areas corresponding to the interictal seizure EEG focus. In some patients, PET has been able to detect a focus when other methods have failed. Studies of patients during partial seizures have shown a change from hypo to hypermetabolism at the site of the focus. In the Lennox-Gastaut syndrome, PET has revealed the existence of two separate metabolic patterns despite clinical seizure similarity.

PET studies allow more definitive overall identification of the epileptic lesion and suggest new avenues of investigation into the basic mechanisms of the epilepsies. MRI may show small structural lesions underlying PET hypometabolism even when CT is normal. Further studies will elucidate the relation of metabolic and pathologic changes. MEG may have the potential to accurately localize the subsurface origin of spikes. EEG provides little information on the spatial distribution of epileptiform in cortical depths; MEEG may be superior.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02562-03 MNB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Neurophysiological Bases of Phonatory Pathology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Christy Ludlow, Ph.D.	Speech Pathologist	SPU,HMCS,MNB,NINCDS
Others:	Nadine P. Connor, M.A.	Speech Pathologist	SPU,HMCS,MNB,NINCDS
	Celia J. Bassich, M.A.	Speech Pathologist	SPU,HMCS,MNB,NINCDS
	Ralph F. Naunton, M.D.	Otolaryngologist	CDP,NINCDS
	Michael Baker, M.D.	Neurologist	OCD,NINCDS
	Young J. Lee, Ph.D.	Statistician	OBFS,NINCDS

COOPERATING UNITS (if any)
EMG Laboratory, OCD NINCDS, BFSB NINCDS, CDP NINCDS

LAB/BRANCH
Medical Neurology Branch

SECTION
Human Motor Control

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	.90	1.00

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose is to determine the neurophysiological bases of phonatory control and its disorders. Acoustic analyses of phonatory signals are aimed at determining how phonation is altered by different pathologies. Initial studies compared groups of patients and found that jitter was increased in morphological changes in the vocal folds while neurological disturbances increased shimmer and not jitter. When individual data were examined to identify acoustic measures sensitive to different laryngeal pathologies, a great deal of overlap between normal and pathologic cases was found on jitter and shimmer. Patients with similar laryngeal disorders performed differently suggesting considerable variation between individuals in controlling laryngeal function.

Acoustic measures have been developed to study the effects of involuntary vocal fold movements, observed fiberoptically, on phonation. Linear variations in frequency were associated with essential tremor of the vocal folds and were not found in other movement disorders. Further studies are planned to examine the acoustic effects of respiratory tremor, vocal tract tremor and vocal fold spasms. The results will assist in understanding neurophysiological control of phonation.

In spasmodic dysphonia, an earlier study demonstrated that phonatory initiation was the speech task most affected in these patients. Subsequently, the coordination and timing between laryngeal and respiratory movements during phonatory initiation and whisper were examined. Slow reaction times and incoordination were found only during phonation, demonstrating task specific voluntary movement difficulties, commensurate with a focal dystonia. EMG studies of the intrinsic laryngeal muscles during speech tasks are aimed at determining the neurophysiological mechansims underlying this movement disorder. This project incorporates previous projects Z01 NS 02440-05 and Z01 NS 02561-02.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02563-03 MNB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Independent Aspects of Speech Timing in Neurological Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Christy Ludlow, Ph.D.	Speech Pathologist	SPU,HMCS,MNB,NINCDS
Others:	Celia J. Bassich, M.A.	Speech Pathologist	SPU,HMCS,MNB,NINCDS
	Nadine P. Connor, M.A.	Speech Pathologist	SPU,HMCS,MNB,NINCDS
	Geralyn Schulz, M.A.	Speech Pathologist	SPU,HMCS,MNB,NINCDS

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Medical Neurology Branch

SECTION
Human Motor Control

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.95	.95	0

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose is to determine which aspects of speech production timing are independently controlled by the central nervous system and the structures controlling them. Patients with neurological disorders/diseases affecting a particular brain region are examined on experimental tasks manipulating separate aspects of speech timing including: syllable initiation time; syllable execution time; alteration of speech in sentences and syllable repetition rate. A series of studies is ongoing which demonstrate that although these aspects of speech timing are inter-related in normal speakers, they are independently affected in different neurological diseases. The results suggest that change in execution time is affected in Huntington's disease while change in speaking rate is affected in Parkinson's disease. Syllable initiation time was most affected when the white matter tracts were involved.

Perceptual studies are employed to evaluate the significance of different speech production disorders for intelligibility. Patients' productions of p, b, and g are identified by listeners in a forced choice procedure. Acoustic measures of articulator timing are compared between correctly identified and incorrectly identified patient productions to determine which articulatory errors result in poor intelligibility in dysarthric speech. Single syllable productions of Parkinson patients were highly intelligible with few listener errors although there were significant acoustic differences between patients and controls. These studies will be continued to examine the effects of speech rate on syllable intelligibility and articulatory accuracy.

This project incorporates previous projects Z01 NS 02185-01 and Z01 NS 02557-03.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02564-03 MNB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Relationships between Language and Speech Deficits in Neuropathologies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Christy Ludlow, Ph.D.	Speech Pathologist	SPU,HMCS,MNB,NINCDS
Others:	Grace Yeni-Komshian, Ph.D.	Neurolinguist	Guest Researcher, MNB
	Celia J. Bassich, M.A.	Speech Pathologist	SPU,HMCS,MNB,NINCDS
	Nadine P. Connor, M.A.	Speech Pathologist	SPU,HMCS, MNB, NINCDS
	Geralyn Schulz, M.A.	Speech Pathologist	SPU,HMCS,MNB,NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Medical Neurology Branch

SECTION

Human Motor Control

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.70	.70	0

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose is to determine which aspects of speech production, speech perception and language are independently controlled by the central nervous system and the brain regions associated with each. To determine at what level in the central nervous system speech motor control is independent of language and speech perception, patients with neurological diseases affecting only one aspect of speech and language are studied, and the location of their neuropathology determined. In one study, patients with normal speech production and language processing following penetrating head injury were examined on experimental tests of speech discrimination and identification. Two groups of patients were identified; one with speech discrimination deficits and the other without such deficits. CT scan data comparisons between the two groups suggested that the upper levels of white matter tracts in either hemisphere, and not the cortical regions, were associated with speech discrimination deficits. These data suggest that speech sound discrimination skills can be affected independently from speech sound identification, language and speech production skills, and are not associated with left sided damage.

A case of chronic cortical deafness with normal speech secondary to herpes encephalitis was studied. Normal brain stem potentials with severely reduced cortical responses indicated that auditory signals were not reaching the cortex. The patient's phonological information processing, speech perception and speech repetition were only mildly impaired in contrast with a central deafness and severe auditory agnosia. This case demonstrates the independence of speech perception and phonological processing from auditory perception.

Data analyses are ongoing for studies of penetrating head injuries to examine the independence of various speech production deficits and the associated lesion locations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02667-01 MNB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physiological Analysis of Involuntary Movements

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Mark Hallett, M.D.	Clinical Director Chief		OCD	ODIR	IRP	NINCDS
Others:	John Ravits, M.D.	Medical Staff Fellow		OCD	ODIR	IRP	NINCDS
	Michael Baker, M.D.	Medical Staff Fellow		OCD	ODIR	IRP	NINCDS
	Leonardo Cohen, M.D.	Visiting Associate	MDU	HMCS	MNB	IRP	NINCDS
	John Schwankhaus, M.D.	Senior Staff Fellow	MDU	HMCS	MNB	IRP	NINCDS
	A. Robert Spitzer, M.D.	Medical Staff Fellow	MDU	HMCS	MNB	IRP	NINCDS
	Jerome Sanes, Ph.D.	Senior Staff Fellow	MDU	HMCS	MNB	IRP	NINCDS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Medical Neurology Branch, Intramural Research Program

SECTION

Movement Disorders Unit, Human Motor Control Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Myoclonus and a number of other rapid involuntary movements have been difficult to classify clinically. Consolidation of patient material obtained outside of NIH with continuing analysis of new cases at NIH has led to new classifications and pathophysiological insights. Conclusions have been drawn about myoclonus of epileptic nature, adult onset tic and startle.

Tremors are also difficult to classify. In studying the physiological aspects of these disorders, we have identified a new descriptor of cerebellar postural tremor which may help distinguish it from other postural action tremors. The amplitude of cerebellar postural tremor appears to depend on the precise posture of the limbs, and, in particular, is highest when the arms are near the body and the hands are pointing toward each other. The physiological implications of this are being explored. We have also studied several patients with orthostatic tremor, a curious, newly described disorder of tremor of the legs only when standing. Our preliminary studies suggest that this is a disorder of the balance mechanism.

We plan to continue these studies and add PET scanning as a physiological tool to our battery of clinical neurophysiological methods to help identify abnormal areas in the brains of patients with involuntary movements.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02668-01 MNB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Trial of Isoniazid for Action Tremor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Mark Hallett, M.D.	Clinical Director Chief		OCD	ODIR	IRP	NINCDS
Others:	John Ravits, M.D.	Medical Staff Fellow		OCD	ODIR	IRP	NINCDS
	Michael Baker, M.D.	Medical Staff Fellow		OCD	ODIR	IRP	NINCDS
	Jerome Sanes, Ph.D.	Senior Staff Fellow	MDU	HMCS	MNB	IRP	NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Medical Neurology Branch, Intramural Research Program

SECTION

Movement Disorders Unit, Human Motor Control Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previous studies have shown utility of isoniazid for ameliorating cerebellar postural tremor in patients with multiple sclerosis. Current studies are aimed at identifying whether patients with action tremors of other types are also benefitted. A double-blind placebo-controlled, cross-over trial is in progress.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	**PROJECT NUMBER** Z01 NS 02669-01 MNB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Physiological Analysis of Voluntary Movement

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Mark Hallett, M.D.	Clinical Director Chief		OCD	ODIR	IRP	NINCDS
Others:	John Ravits, M.D.	Medical Staff Fellow		OCD	ODIR	IRP	NINCDS
	Michael Baker, M.D.	Medical Staff Fellow		OCD	ODIR	IRP	NINCDS
	Leonardo Cohen, M.D.	Visiting Associate	MDU	HMCS	MNB	IRP	NINCDS
	John Schwankhaus, M.D.	Senior Staff Fellow	MDU	HMCS	MNB	IRP	NINCDS
	A. Robert Spitzer, M.D.	Medical Staff Fellow	MDU	HMCS	MNB	IRP	NINCDS
	Jerome Sanes, Ph.D.	Senior Staff Fellow	MDU	HMCS	MNB	IRP	NINCDS

COOPERATING UNITS *(if any)*
Walter Reed Army Hospital, Department of Neurology (Vietnam Head Injury Study)
Drs. Andres Salazar and Stephen C. Vance

LAB/BRANCH
Medical Neurology Branch, Intramural Research Program

SECTION
Movement Disorders Unit, Human Motor Control Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A laboratory is being set up for analysis of balance. This will enable simultaneous study of body angles, foot-floor forces and multiple EMGs. We hope to identify normal mechanisms of balance and how these are deranged in patients.

The study of motor control in hemiplegia is being set up as the major project of the Unit. Patients with discrete brain lesions will be studied; patients with strokes will be the main group, and many patients will be followed serially from onset of the disorder to recovery. We hope to discover pathophysiological mechanisms including those mechanisms underlying recovery. Methods of study will include (1) quantitative clinical battery, (2) evoked potentials, (3) pre-motor potentials, (4) stretch reflexes, (5) EMG analysis of voluntary movements, and (6) PET scanning during voluntary movement.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02630-02 MNB

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical, Genetic, and Biochemical Studies of Familial Alzheimer Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Ronald J. Polinsky, Chief, CNS, MNB, NINCDS
Linda E. Nee, OCD, NINCDS
Robert T. Brown, CNS, MNB, NINCDS
James Gusella, Genetics Unit, Dept. of Neurology, Mass. Gen. Hosp, Boston, MA
Michael Conneally, Dept. of Genetics, Indiana University
Luigi Amaducci, Dept. of Neurology, Univ. of Florence, Italy
Jean-Francois Foncin, Laboratory of Histopathology, La Salpetriere, Paris, France
Herbert Weingartner, Laboratory of Neuropsychology, NIMH

COOPERATING UNITS (if any) Laboratory of Histopathology, La Salpetriere, Paris, France; Office of the Clinical Director, NINCDS; Laboratory of Neuropsychology, NIMH; Genetics Unit, Dept. of Neurology, Mass. Gen. Hosp, Boston, MA; Dept. of Genetics, Indiana University; Dept. of Neurology, Univ. of Florence, Italy

LAB/BRANCH

Medical Neurology Branch, IRP, NINCDS

SECTION

Clinical Neuropharmacology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Alzheimer's disease is the most common cause of irreversible, chronic dementia. One factor which complicates the interpretation of many clinical research studies is that 20% or more of clinically diagnosed cases do not have Alzheimer's disease at autopsy. Although Alzheimer's disease may be inherited in less than 10-20% of all cases, the main justification for studying familial cases lies in the accuracy of diagnosis which may be inferred through post-mortem examination of other affected family members.

Previous genetic studies have not clarified the role of inheritance. Recent advances in the field of molecular biology have resulted in the development of recombinant DNA technology. Other molecular approaches that are being used to study degenerative neurological disorders include investigations of DNA repair, immunological function and abnormal protein production. In this project skin fibroblast and peripheral blood lymphoblast cultures will be established from members of large kindreds with familial Alzheimer's disease. These cultures will serve as a renewable source of DNA and cell lines which can be used for genetic linkage, viability, and biochemical studies.

Alzheimer's disease may result from a form of primary neuronal degeneration. Neurotransmitter studies suggest that there is a central nervous system degeneration of cholinergic neurons. However, there is substantial evidence which shows that the locus ceruleus, an important noradrenergic nucleus, is also involved as well as other neurotransmitter and peptide systems. In order to define the natural history, temporal progression, and biochemical abnormalities in Alzheimer's disease, this project will include a longitudinal study of affected and at-risk subjects from large kindreds with familial Alzheimer's disease. Detailed neuropsychological testing, PET scanning, neurotransmitter studies, and pharmacological investigations are planned. Neuropathological and neurochemical studies of post-mortem specimens from these families will also be conducted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02115-12 MNB

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Indices of Adrenergic Function in Humans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Ronald J. Polinsky, Chief, CNS, MNB, NINCDS
Robert T. Brown, CNS, MNB, NINCDS
Lillian Recant, Division of Endocrinology, VA Hospital, Washington, DC
Linda E. Nee, OCD, NINCDS
Giovanni DiChiro, NIS, OD, IRP, NINCDS
Berham Pastakia, Diagnostic Radiology, Clinical Center
Richard S. Burns, OD, IRP, NINCDS
David S. Goldstein, Hypertension-Endocrine Branch, NHLBI

COOPERATING UNITS (if any)

OCD, NINCDS; Neuroimaging Section, IRP, OD, NINCDS; Diagnostic Radiology, CC; Division of Endocrinology, VA Hospital, Washington, DC; OD, IRP, NINCDS; Hypertension-Endocrine Branch, NHLBI

LAB/BRANCH

Medical Neurology Branch, IRP, NINCDS

SECTION

Clinical Neuropharmacology Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Autonomic nervous system activity is essential for maintaining circulatory and metabolic homeostasis. In order to study sympathetic nervous system function and its relationship to other neuroendocrine systems, it is necessary to measure neurotransmitter, hormonal, and peptide levels in response to various stimuli. The levels of norepinephrine, epinephrine, and dopamine and their metabolites in various body fluids reflect the activity of the neurones from which these neurotransmitters are released. Although plasma levels of norepinephrine reflect the responses of the peripheral sympathetic nervous system it is necessary to consider removal rates of the catecholamine. Measurement of urinary catecholamine metabolites and their stereospecific labelling pattern following administration of radiolabelled isomers of norepinephrine provides a means for investigating intraneuronal norepinephrine metabolism. Cerebrospinal fluid levels of monoamine metabolites can be used to assess central nervous system neurotransmitter metabolism. It is necessary to consider the origin of these metabolites to make appropriate corrections for valid interpretations of the data. These strategies have been used to study patients with neurogenic orthostatic hypotension and in other clinical situations in which adrenergic function is abnormal. Investigation of the effects of aging on autonomic nervous system function is in progress. A more thorough understanding of neurotransmitter metabolism in these clinical situations leads to more rational approaches to therapy.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01658-18 MNB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Hemispheric Development and Specialization of the Intellectual Functions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. Fedio	Psychologist	MN	NINCDS
	P. Brouwers	Psychologist	LPP	NIMH
Other:	A. Martin	Psychologist	MN	NINCDS
	C. Cox	Psychologist	MN	NINCDS
	W. Meyer	Medical Officer	SN	NINCDS
	C. Kufta	Medical Officer	SN	NINCDS

COOPERATING UNITS (if any)

Surgical Neurology Branch, IRP, NINCDS
Laboratory of Psychology and Psychopathology, IRP, NIMH

LAB/BRANCH

Medical Neurology, IRP, NINCDS

SECTION

Clinical Neuropsychology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	0.6	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The disabling effects of chronic cerebral insult and neuropsychiatric disorders were evaluated by a broad range of neuropsychological tests evaluating brain-behavior relations in man.

Asymptomatic long term survivors of acute lymphoblastic leukemia (ALL) who received CNS preventive therapy (cranial irradiation and intrathecal chemotherapy) were studied. Based on CT scan findings, the patients were divided into three groups: normal scans, cortical atrophy; intracerebral calcifications. Memory and learning were significantly impaired in children with abnormal scans, more so for the patients with calcification. In addition, all patients with abnormal CT scans showed significant attentional dysfunctions.

Adolescents with obsessive compulsive features exhibited a cluster of neuropsychological deficits which correlated with ventricular enlargement. Deficits were identified in spatial judgement and spatial learning. It was suggested that an imbalance in the inhibitory functions of the frontal lobe and limbic systems may contribute to obsessive compulsive behavior.

Patients with Tourette's Syndrome were evaluated in an effort to characterize the neuropsychological defects in frontal inhibitory mechanisms. Positron emission tomographic (PET) data of regional cerebral activity from these patients will be correlated with test performance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS0 1424-19 MNB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Behavioral Modulation by the Limbic System in Man

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. Fedio	Psychologist	MN	NINCDS
	A. Martin	Psychologist	MN	NINCDS
Other:	P. Brouwers	Psychologist	LPP	NIMH
	C. Cox	Psychologist	MN	NINCDS
	F. Lalonde	Psychologist	MN	NINCDS
	E. Mohr	Psychologist	MN	NINCDS
	E. Witt	Psychologist	MN	NINCDS
	C. Kufta	Medical Officer	SN	NINCDS

COOPERATING UNITS *(if any)*
Surgical Neurology Branch, IRP, NINCDS
Laboratory of Psychology and Psychopathology, IRP, NIMH

LAB/BRANCH
Medical Neurology, IRP, NINCDS

SECTION
Clinical Neuropsychology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Emotional and cognitive characteristics were studied in epileptic patients following unilateral left or right temporal lobe resection. The integrity of attentional and perceptual (visual, auditory, and tactile) systems were evaluated using standard and experimental procedures. Physiological events (skin conductance) were monitored and recorded during test performance. The research examined the role of the temporal lobe in establishing specific limbic associations between left and right hemispheres in regulating cognitive functions and emotional experiences in man.

Tachistoscopic studies identified a critical perceptual role for right temporal mechanisms, especially during the early stages of visual processing. The left and right temporal lobes contribute differentially to specifying the identity of a stimulus, but not to its position or orientation in space. Left temporal mechanisms encode verbal information during initial learning. Modest compensation for memory defects following temporal lobectomy may be achieved with strategy which combines overt and covert imagery with praxic encoding.

In affective sectors, left temporal patients tend to neutralize reactions to nuances with emotional coloration; right temporal patients, in contrast, rate these materials similar to normal individuals. Unlike normal individuals, however, the left and right temporal lobectomy patients were hyporesponsive to affective material as indexed by skin conductant measures. Moreover, both lobectomy groups failed to take advantage of the emotional characteristics of information to facilitate memory. These data suggest that unilateral temporal lobectomy disrupts the normal linkage of cognitive-affective associations mediated by temporal limbic interaction. There were, however, beneficial effects to surgical treatment in that patients, following temporal lobe surgery, were less deviant from normal subjects in emotional behavior.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS0 1245-20 MNB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

EEG Learning Correlates Using Scalp and Intracranial Depth Electrodes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Johnson	Psychologist	MN	NINCDS
	P. Fedio	Psychologist	MN	NINCDS
Other:	A. Martin	Psychologist	MN	NINCDS
	C. Kufta	Medical Officer	SN	NINCDS
	P. Brouwers	Psychologist	LPP	NIMH

COOPERATING UNITS *(if any)*

Surgical Neurology Branch, IRP, NINCDS
Laboratory of Psychology and Psychopathology, IRP, NIMH

LAB/BRANCH

Medical Neurology, IRP, NINCDS

SECTION

Clinical Neuropsychology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Information processing was monitored and quantified by averaged evoked response techniques. The electrographic activity was recorded from left and right brain regions during memory and perception in normal subjects, patients with unilateral temporal lobectomy, and patients with neuropsychiatric disorders. Electroencephalographic disturbances in brain-behavior relations in psychiatric patients were also evaluated, relating left brain dysfunction to ideational disorders, and right brain activity to maladaptive emotional reactions.

In temporal lobectomy patients, P300 amplitude was found to be inversely proportional to stimulus probability in the same way as for normal controls, and larger P300s were elicited in reaction time. For visual material, right temporal patients manifested smaller P300s at frontal sites than either left temporal or normal individuals. Moreover, there were no consistent hemispheric asymmetries which distinguished the left or right temporal patients, or either group from normal subjects. These data discount the hypothesis that medial temporal structures, including the hippocampus, serve as a sole generator of P300. More specifically the data indicate that processing of auditory and visual stimuli is dependent to a great extent on the character of the material and the integrity of left and right brain mechanisms.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 00200-31 MNB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cognitive and Emotional Profile of Neuropsychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. Fedio	Psychologist	MNB	NINCDS
	A. Martin	Psychologist	MNB	NINCDS
Other:	P. Brouwers	Psychologist	LPP	NIMH
	C. Cox	Psychologist	MNB	NINCDS
	F. Lalonde	Psychologist	MNB	NINCDS
	E. Mohr	Psychologist	MNB	NINCDS
	E. Witt	Psychologist	MNB	NINCDS
	T. Chase	Neurologist	ET	NINCDS

COOPERATING UNITS (if any)

Experimental Therapeutics Branch, IRP, NINCDS
Laboratory of Psychology and Psychopathology, IRP, NIMH

LAB/BRANCH

Medical Neurology, IRP, NINCDS

SECTION

Clinical Neuropsychology

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A neuropsychological profile of dementia was drafted for individuals with Alzheimer's Disease, Huntington's Disease and 'at risk' for Huntington's Disease. The evaluations extended into memory, learning and perceptual areas, utilizing standard and experimental tasks, also establishing normative references for functional changes accompanying the aging processes.

Although Alzheimer's Disease is accompanied by marked deficits in selective attention, memory and learning, there were no qualitative differences between demented and age-matched subjects. The impairment also extended to object-naming and fluency, and AD patients performed poorly in perceiving meaning, except when the stimuli required emotional judgement. The data indicate that Alzheimer's patients may be unable to encode material; this is in sharp contrast with other amnesic disorders where the primary difficulty involves an inability to store and/or retrieve information.

Alzheimer's and Huntington's patients showed pronounced but dissimiliar deficits with visuospatial and constructional tasks. The behavioral data extend neuropathologic impressions of degeneration of the frontal striatal system in Huntington's Disease, and temporo-parietal, cortical involvement in Alzheimer's Disease.

The neuropsychological test profile of Alzheimer's patients yielded different clinical subgroups or populations. Memory and learning deficits, per se, were poor indicators of group membership. One group was characterized by severely impaired verbal abilities, but with intact perceptual and constructional skills. The second group was more impaired on perceptuomotor than verbal tasks. The third group showed comparable deficiencies in both linguistic and visual spatial sectors. Positron emission tomographic and EEG data confirmed corresponding changes in left, right or bilateral regions in the posterior cerebral quadrant, respectively.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02678-01 MNB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Involvement of Calcium, Fodrin, and Glutamate in Seizures

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. Suzan Nadi, Ph.D. Senior Staff Fellow CES, MNB, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Medical Neurology Branch, Intramural Research Program

SECTION
Neuronal Excitability

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.05	0.15

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The work will involve the study of epileptic foci and the evaluation of the status of glutamate receptors, the cytoskeletal protein fodrin and the flux of calcium ions. The epileptic seizure has been demonstrated to occur as a result of a shift of external calcium ions to the interior of the cell. Since calcium ions are also known to activate calpain, which degrades fodrin, which is responsible for maintaining the integrity of the membrane. The question of interest in this study is to determine whether fodrin breakdown is directly linked to the spread of epileptic activity.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER 201 NS 02679-01 MNB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Plasma and CSF Levels of Neurotransmitters in Epileptics and to EKG Correlation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Mark Holmes, M.D., Medical Staff Fellow, CES, MNB, NINCDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Medical Neurology Branch, Intramural Research Program

SECTION
Neuronal Excitability

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.05	0.15

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Blood and CSF samples will be obtained from patients with epilepsy before and after a seizure. Neurotransmitters such as catecholamines, amino acids, and peptides will be measured by HPLC and RIA techniques. These findings will be correlated to any EEG or EKG changes which occur during seizures in order that some insight into the sudden death syndrome in epilepsy might be obtained.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02269-09 MNB

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Visual Evoked Potentials in Clinical Neurology and Neuro-Ophthalmology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Susumu Sato, M.D.	Medical Officer	MNB, NINCDS
OTHERS:	Douglas F. Rose, M.D.	Medical Staff Fellow	OCD, NINCDS
	Vita Alexander, REEGT	Chief Technologist	OCD, NINCDS
	William Thomas	EEG Technologist	OCD, NINCDS

COOPERATING UNITS *(if any)*

Office of Clinical Director, IRP, NINCDS

LAB/BRANCH

Medical Neurology Branch, IRP, NINCDS

SECTION

Neuronal Exitability Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Visual evoked potentials to checkerboard pattern, were studies in normal volunteers and patients with various neurological disorders, particularly multiple sclerosis and seizures.

A. Multiple Sclerosis: The prolongation of the major positive peak has been consistently found in patients with history of optic neuritis and in some patients even without such a history. In some patients with the history of optic neuritis who have been visually asymptomatic for many years, however, persistent prolongation or normalization of the latency has been noted.

B. Epileptic Seizures: Visual evoked potentials to be a half-visual field stimulation (studying the retrodiasmatic visual pathway) have been studied in patients with complex partial seizures. The primary goal is to predict the side of the epileptic lesion by the visual evoked potentials. The preliminary analysis in 6 patients showed no clear-cut predictability.

C. Other Neurological Disorders: Visual evoked potentials were studied in a patient with Shapiro syndrome who has paroxysmal hypothermia. When the body temperature dropped to 33-34°C, the latency of the major positive peak was significantly prolonged, whereas with normal body temperature (35-36°C), the latencies were normal.

The significance of the visual evoked potentials lies in the fact that they are totally noninvasive, are useful in detecting the occult lesions and in evaluating the visual system in the context of the cortical nervous system integrity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02431-06 MNB

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Experimental Epilepsy: Seizures Produced by Kindling in Rat

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Shun-ichi Yamaguchi, Ph.D.	Psychologist	MNB, NINCDS
OTHERS:	Susumu Sato, M.D.	Medical Officer	MNB, NINCDS
	Stuart Walbridge	Lab Specialist	MNB, NINCDS

COOPERATING UNITS *(if any)*

Office of the Clinical Director, IRP, NINCDS

LAB/BRANCH

Medical Neurology Branch, IRP, NINCDS

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.6	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In rats, seizures produced by chronic stimulation (Kindling) of amygdaloid complex or other nuclei were studied.

A. Kindling and heart rate changes: Arrythmia of the heart rate was noted with kindled seizures. However, the kindled seizures after paralyzing rats with a muscle relaxant (Flaxedil) did not produce the heart rate change. The results suggested that not only the central but also the peripheral mechanism (intact chest cage) might be associated with irregularity of heart rate.

B. Seizure patterns produced by caudate and globus pallidus kindlings: No significant difference existed between the caudate and the globus pallidus animals in kindling rate, but they were somewhat different from and required twice as may stimulations as the amygdala rats. Seizures in the globus pallidus rats consisted of initial rotation toward the stimulated side, then after righting, chewing, forelimb clonus, rearing and falling. The caudate seizure started with initial opisthotonic posturing, then maintaining the posture, chewing and forelimb clonus. The results suggested that these nuclei were mostly a relay station for seizure propagation.

C. Effects of neonatal hypoxia or kindling in early adulthood: There was no significant difference in kindling spread and length of afterdischarges between control rats and those exposed to hypoxia.

The significance of the experiments lies in the fact that seizures produced by kindling are a good model for human epilepsy and that the further understanding of kindled seizures will in turn elucidate the mechanism of human epilepsy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02432-06 MNB

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brainstem Auditory Evoked Potentials in Clinical Neurology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Susumu Sato, M.D.	Medical Officer	MNB, NINCDS
OTHERS:	Douglas F. Rose, M.D.	Medical Staff Fellow	OCD, NINCDS
	Vita Alexander, REEGT	Chief Technologist	OCD, NINCDS

COOPERATING UNITS (if any)

Office of the Clinical Director, IRP, NINCDS

LAB/BRANCH

Medical Neurology Branch, IRP, NINCDS

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Brainstem auditory evoked responses to clicks were studied in normal volunteers and in patients with various neurological disorders.

A. Multiple Sclerosis: In some patients with multiple sclerosis, prolongation of latencies, distortion of wave forms or disappearance of some components were noted.

B. Epilepsy: In patients with complex partial seizures no significant changes in terms of latencies or waveform have been noted.

C. Other Neurological Disorders: In a patient with Shapiro syndrome who had paroxysmal hypothermia, the latencies were prolonged when the core temperature dropped to 32-34°C, but returned to normal when the temperature was up to 35-36°C.

The significance of this project is that it can provide information on the functional integrity of the brainstem.

TAB 18 -- NEUROEPIDEMIOLOGY BRANCH -- (NEB)

ANNUAL REPORT

October 1, 1984 through September 30, 1985

Neuroepidemiology Branch

ational Institute of Neurological and Communicative Disorders and Stroke

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Annual Report
for Period October 1, 1984 through September 30, 1985
Neuroepidemiology Branch
Intramural Research Program
National Institute of Neurological and Communicative
Disorders and Stroke

Bruce S. Schoenberg, M.D., Dr.P.H., Chief

The Neuroepidemiology Branch is responsible for the development and implementation of epidemiologic and genetic programs to investigate the cause, prevention, and treatment of neurologic disorders in human populations. Emphasis has been placed on major neurologic diseases in which the diagnoses can be clinically verified to the satisfaction of skilled neurologists. The Branch is unique in being the only unit devoted exclusively to research in the epidemiology of diseases of the nervous system.

Neuroepidemiologic research studies require collaboration of many individuals. However, since there is a severe shortage of available manpower in neuroepidemiology, the Branch has developed an active teaching program for current and future collaborative investigators. A series of six videotapes produced by the Branch are distributed on a loan basis without charge. A textbook, entitled NEUROLOGICAL EPIDEMIOLOGY: PRINCIPLES AND CLINICAL APPLICATIONS, has been prepared. In cooperation with the World Health Organization and the World Federation of Neurology Research Committee on Neuroepidemiology, formal courses were conducted in Caracas, Venezuela, Shanghai, the People's Republic of China, Nijmegen, the Netherlands, and Bombay, India. Additional courses will be held in Jerusalem, Israel, and Hamburg, West Germany. We are also providing opportunities for fellows to spend from six months to two years working with members of the Branch in order to learn the techniques of neuroepidemiology. During the past year we have had physicians from Ecuador, India, Italy, the People's Republic of China, Costa Rica, and Canada, and have received inquiries from Tunisia, and Israel for future opportunities. Finally, current individual and institutional research training grant programs have been expanded to include neuroepidemiology. Institutional grants for training in neuroepidemiology have been awarded to Columbia University, New York, the University of California at Los Angeles, and Temple University, Philadelphia.

To further stimulate neuroepidemiologic research on a worldwide basis, the Branch organized scientific sessions where many investigators presented their work. A meeting of the Research Committee on Neuroepidemiology of the World Federation of Neurology was organized by our Branch in Dallas, Texas. Representatives from Colombia, Ecuador, Italy, the U.S., Canada and Venezuela attended the session and presented data based on a uniform protocol. A workshop was held in Bethesda to plan research strategy to investigate the problem of spastic paraparesis in different parts of the world. This is a significant problem in Colombia, India, the Seychelles Islands, and the West Indies. Further studies are planned in these countries. A large study of mental retardation is being planned using a uniform protocol for implementation in Ecuador, India, and the People's Republic of China. Neuroepidemiology has been selected as one of the four main themes for the next World Congress of Neurology to be held in Hamburg, West Germany in 1985. These sessions serve as a stimulus for neuroepidemiologic research on a worldwide basis.

Epidemiologic studies have two basic requirements: uniformity and accuracy of data collection. This necessitates the use of a standardized, internationally accepted classification and coding system. The currently available scheme published by the World Health Organization is seriously deficient with regard to neurologic disorders. The Branch is therefore collaborating with the World Health Organization Neurosciences Program, the World Federation of Neurology, and the American Academy of Neurology to revise this system of classification and improve its usefulness for neuroepidemiologic research. Two members of the Branch were selected to serve on the advisory committee to the World Health Organization to make recommendations for changes in this classification. A draft of the proposed changes has been prepared and will be circulated to neuroscientists from around the world for comments. However, since this new classification will not be available until 1992, the currently available scheme has been modified to make it more suitable for worldwide research in neuroepidemiology. This scheme will be published by the World Health Organization in 1985.

Another important problem for the neuroepidemiologist is the enormous cost of maintaining neurologic surveillance on a large number of patients. Therefore, we have attempted to utilize existing registries of neurologic disease, such as in a study of presenile dementia based on the Israeli National Neurologic Disease Registry. In addition, we have assisted British investigators in organizing information routinely collected through the British National Health Service on all neurologic inpatients in a section of London with a population of 3-1/2 million inhabitants. The utility and accuracy of these data have been demonstrated in a study of the Guillain-Barré syndrome. A similar registry is being organized for the population of northeastern Italy. We also collaborate with the Mayo Clinic in Rochester and utilize their record-linkage system to study neurologic diseases in the population of Rochester, MN.

There have been a number of neuroepidemiologic case-control studies which have suggested associations between a given factor and a particular disease, but the number of patients has been inadequate for meaningful conclusions. We are working in collaboration with a number of clinical units in Italy to conduct case-control studies of clinically diagnosed cases of Alzheimer's disease and the Burke Rehabilitation Center in White Plains, New York. Similar arrangements have been made to work in conjunction with the Alzheimer's Disease and Related Disorders Association. The first study in collaboration with this Association is currently in progress in Denver, Colorado.

With regard to neurologic problems in children, the Branch documented the frequency of primary intracranial neoplasms in the pediatric population of Rochester, MN, and the State of Connecticut. In addition, we investigated cerebrovascular disease in infants and children. The magnitude of this problem was documented for the first time. The study demonstrated that neonatal intracranial hemorrhage is relatively common (1.1 cases/1,000 live births), that it is strongly associated with prematurity and hyaline membrane disease, and that it is difficult to recognize clinically. For pediatric cerebrovascular disease unassociated with birth, trauma, or infection, the incidence rate was 2.5/100,000/year. These cases were further characterized by survival, residual disability, and cause (whenever possible). The clinical and angiographic features of children with moyamoya disease were examined in detail. This condition appears to be more common than suggested by early case reports.

The Branch is also investigating the epidemiology of cerebral palsy (CP). A study of temporal trends in the incidence rate of CP for Rochester, MN, addressed the concern that advances in perinatal care, by rescuing the compromised neonate, are increasing the rate of neurologic handicap. All identified cases of CP born to Rochester residents during a 27-year period were studied. The overall incidence rate of CP declined from 2.3 to 1.6 cases per 1,000 neonatal survivors. Correlation of birthweight-specific rates of neonatal mortality and CP incidence showed that for the low birthweight neonate, coincident with a marked drop in mortality, the CP incidence rate remained unchanged. For the newborn with birthweight over 2500 g., the rates of CP incidence and neonatal mortality declined in parallel. In a study of CP outcome, a decreased survival was limited to individuals who needed custodial or total nursing care. For the remainder of the case sample, all survived a minimum of 10 years, and in several of the cases there was resolution of the motor handicap.

Studies of neonatal mortality were initiated by the Branch because antecedents of pre- and perinatally incurred neurologic handicap and those of neonatal death overlap. While uniform and complete case identification in a large population over a long period of time is not available for CP, infant birth/death certificate linkage provides such case identification for neonatal death. Using the infant birth/death file of the State of Minnesota, the Branch is now completing two descriptive investigations of neonatal mortality: 1) delineation of neonatal mortality rates (NMR) by sex in gestational age/birthweight-specific subgroups for years 1970-1980, and 2) a study of sex- and birthweight-specific NMR trends for years 1967-1976. The future objective of both the Rochester CP incidence study and Minnesota NMR study is to conduct case-control studies in search of maternal, fetal, and obstetric risk factors of CP and of neonatal death.

The Branch has conducted extensive investigations of primary intracranial neoplasms. First, problems with nomenclature and disease definition were resolved. A number of descriptive studies were carried out, revealing two patterns of age-specific incidence. Analyses of most population-based data worldwide demonstrated a small childhood peak, followed by a later peak between ages 50 and 80. Data for Rochester, MN, however, showed the childhood peak, followed by an increasing incidence rate with increasing age. Careful study of this discrepancy showed 1) that the greater percentage of cases first diagnosed at autopsy in Rochester accounted in large part for this difference, and 2) that a substantial number of brain tumors remain undiagnosed in the elderly during life. Studies have just been completed to evaluate the role of computerized tomography in the diagnosis of brain tumors and to explain the recent increase in the incidence of pituitary tumors among women of childbearing age. The introduction of computerized tomography has not resulted in any increase in the reported frequency of these tumors in the Rochester, MN population, while the apparent rise in the incidence of pituitary tumors seems to be the result of more sophisticated neuroendocrine diagnostic procedures. A comprehensive study of U.S. and international mortality data for primary nervous system neoplasms over a 15-25 year period demonstrated an increasing death rate, especially among the elderly. This was thought to be due to improved diagnosis and case ascertainment. An exhaustive, critical review of a survey strategy to measure the national incidence and prevalence of intracranial neoplasms has been completed. In addition, racial differentials in the frequency of certain intracranial tumors (meningiomas and pituitary adenomas) are being examined. Investigations of the relationship between intracranial neoplasms and extracranial tumors have been especially rewarding. An association was found between the occurrence of breast cancer and meningioma in women. This result raises interesting etiologic possibilities when considered with other evidence: 1) meningioma is the only common intracranial neoplasm with a higher incidence in females; 2) the abrupt clinical appearance or enlargement of this tumor during pregnancy has been described; and 3) the finding of estrogen receptor protein in meningioma has been reported. To identify possible risk factors for specific types of tumors, a case-control study is being conducted of glioblastoma multiforme.

Epilepsy is a major cause of morbidity both in the U.S. and other parts of the world. Thus, scientists in the Branch are conducting many studies on epilepsy. The record-linkage system for Rochester, MN, has also been used to identify all possible cases of complex partial seizures occurring in the years 1935-1979. A case-control study is being designed to identify risk factors associated with the occurrence of such seizures. This study is in the phase of data analysis and the final results will be presented at the annual meeting of the American Neurological Association in Chicago in October 1985. This study is now being extended to absence seizures, myoclonic seizures and tonic-clonic seizures. Studies to document the prevalence of epilepsy have been conducted in the People's Republic of China, Nigeria, Ecuador. These are now being followed by case-control studies to identify specific risk factors as applicable to different parts of the world. For example, there is some evidence that cerebral cysticercosis the commonest cause of epilepsy in Ecuador, whereas cerebral trauma is an important cause in the U.S.

At the present time, there is little to suggest that improved medical management of the completed stroke will substantially affect the cerebrovascular disease problem. It would appear that greater benefit could be achieved by dealing with the precursors of stroke rather than delaying treatment until after the event has occurred. Therefore, a nonconcurrent, prospective study of a cohort of 2,000 elderly individuals was undertaken to determine the role of heart disease and hypertension as risk factors for both transient ischemic attacks (TIA) and completed stroke. When the case-control approach was applied to these data, different patterns of risk factors were demonstrated for TIAs and completed ischemic stroke. While hypertension, diabetes mellitus, definite hypertensive heart disease, and valvular heart disease are important risk factors for completed ischemic stroke, these disorders do not have a substantial effect on the subsequent risk of TIA. When these data were analyzed in the format of a prospective study, it was possible to calculate the absolute risk of stroke as a function of the presence or absence of specific forms of cardiovascular disease. The following types of cardiovascular disease yielded the highest completed ischemic stroke incidence rates (cases/1,000/year): myocardial infarction (15.5); congestive heart failure (20.5); and TIA (42.0). In considering risk factors for TIA, both angina/coronary insufficiency and congestive heart failure yielded the highest rates (10.4 and 10.9, respectively). Once etiologic precursors of stroke have been identified, medical intervention before the occurrence of long-lasting disability requires that there be an interval of time between the onset of the risk factor and the development of completed stroke. Analysis of data from this nonconcurrent prospective study revealed that those developing borderline hypertension, valvular heart disease, or ischemic heart disease remained stroke-free for the initial one and one-half years after the first occurrence of each specific form of cardiovascular disease. This finding implies that there is an interval of time following the onset of these conditions when it may be possible to intervene medically to reduce the risk of stroke.

Previous studies of stroke incidence have generally utilized one of two techniques: a) survey of an entire community to identify all cases of stroke or b) survey of all community residents hospitalized for stroke in medical institutions serving that population. Rates derived from community surveys are usually higher than those obtained from hospital statistics. To quantify the size of the error inherent in using hospitalized cases, we applied both methodologies to the same population. Cases of completed stroke occurring among residents of Rochester, MN, during 1955-1969 were verified by neurologic review of data from a records-linkage resource. In this community, patients are hospitalized following stroke on the basis of medical necessity. Records for all 993 patients were reviewed to determine whether the patient was admitted to an acute-care hospital for the stroke. Overall, 69% of stroke cases were admitted to an acute-care facility. This study suggests that incidence rates derived from hospital data underestimate the frequency of new strokes by 25-30%; this discrepancy is most marked in the elderly. Another investigation based in this same community studied stroke in patients already hospitalized for other conditions. Sixty-five individuals suffered a first completed stroke while in a short-stay hospital for either a medical problem or surgical procedure. This represents 6.5% of all first strokes in the Rochester population. The percentage of all first completed strokes occurring during a short-stay hospitalization was slightly higher for women (8%) than for men (5%). In 74%, the stroke was directly related to medical conditions or surgical procedures. Etiologic factors preceding stroke, in order of frequency, were acute heart disease (21), major surgical procedures (10), fractures (8), leukemia or blood dyscrasias (5), acute gastrointestinal bleeding (3), and cerebral angiography (1). In the remaining 17 patients without an obvious event or clearly attributable etiologic factor leading to the stroke, all but 5 had either diabetes mellitus, chronic heart disease, or hypertension. There were 99 additional Rochester residents suffering a first completed stroke while in a nursing home or chronic care facility, raising the total strokes in residents of hospitals or nursing homes to 11.5% of all first strokes in the community. Other investigations in the area of stroke involve a careful analysis of unusual patterns of cerebrovascular disease (e.g., more than 20 TIAs/day).

Alzheimer's disease/senile dementia, despite its high apparent clinical frequency among the elderly, has not been well studied in a U.S. population. Thus a major effort is being made by the Branch to study dementia in general and Alzheimer's disease in particular. Three descriptive studies based on well-defined populations have been conducted. One is derived from a review of detailed clinical records utilizing a population-based, records-linkage system. A neurologist using fixed diagnostic criteria reviewed records from all medical facilities serving the residents of Rochester, MN. This made it possible for the first time to determine the incidence of dementia coming to medical attention in a well-defined U.S. population. For those age 30 plus, the incidence rate was 110 new cases/100,000 population/year. The rates increase with age, and the age-specific rates were higher in women. To confirm the reduced survival of demented patients reported on the basis of individuals hospitalized at specific medical centers, we examined the survival of all demented individuals identified through our records-linkage study. Dealing with an entire population minimizes any possible selection bias that may be present for a series of patients seen at a particular medical institution. The survival rates generated for all demented patients in the defined population were significantly reduced compared to age- and sex-matched survival statistics derived from life-tables for residents of the northwest central region of the U.S., thereby documenting in a community study previous observations based on hospitalized patients.

The second investigation, a two-stage survey, permitted us to estimate the prevalence of dementia in a biracial community. For each race, prevalence ratios were higher for females. For each race and sex, the prevalence figures rise dramatically with age. This morbidity study indicates that dementia represents a major health problem for both racial groups.

A third population-based study was conducted in Israel. There has been some debate as to whether Alzheimer's disease is a single disease entity regardless of its age at presentation. Since the frequency of Alzheimer's disease is relatively low before age 60, an enormous population is required for surveillance in order to obtain an adequate number of patients for study. We have therefore utilized the resources available through the Israeli National Neurologic Disease Registry to identify all potential cases among the population of Israel. These cases were intensively reviewed to determine the accuracy of diagnosis and to explore a number of epidemiologic studies of the distribution and risk factors for this disease. A similar pattern has emerged for those age 60 and under as has been described in previous studies for older individuals. The incidence rates increase with age, and the disease is slightly more common in women. Of particular interest is the finding that the risk of early-onset Alzheimer's disease (age 60 years and earlier) is significantly higher among Jews of European-American origin compared to those born in Africa or Asia.

In addition, four case-control studies are in progress. The first utilizes cases and controls selected from the Rochester, MN population. Past medical records have been utilized to obtain information concerning possible associations between Alzheimer's disease and either medical conditions or surgical procedures. Three case-control studies of Alzheimer's disease utilizing interview data are being carried out in conjunction with a) the Alzheimer's Disease and Related Disorders Association, and b) the Italian National Research Council, and c) the Burke Rehabilitation Center in White Plains, New York. The latter three studies are utilizing a similar protocol to enable comparison of results obtained from studies conducted in populations which are widely different. Patients affected by Alzheimer's disease or senile dementia of the Alzheimer's type have been identified by means of a specific protocol employing a defined algorithm. Since most of the patients are unable to give adequate information at the interview because of the mental impairment, a questionnaire for a next-of-kin interview was prepared. The questionnaire attempts to obtain information on various risk factors. The study with the Alzheimer's Disease Association is in the advanced stages of data collection. The study in Italy is complete and a final manuscript is being prepared.

Mortality data for the entire U.S. for various causes of dementia have been studied for the years 1971 and 1973-1978. This study found that a majority of people with dementia died of other causes, suggesting that dementia may contribute to increased mortality indirectly. Many treatable and preventable conditions such as pneumonia and trauma were also found to be associated with patients with dementia at the time of death. Aggressive management of these conditions may increase longevity in some of these patients.

Investigations are in progress to identify familial cases of Alzheimer's disease in the Italian population. These familial cases will be studied in great detail utilizing the latest technology available such as gene mapping. Methodologies for sample collection and transportation have been discussed with the Camden cell-line depository.

Yet another approach will utilize information obtained from clinical examination and combine it with autopsy data, thereby establishing a more definitive diagnosis of Alzheimer's disease. The objective of this study is to highlight the clinical characteristics which are most closely associated with pathologically proven Alzheimer's disease. This should help improve clinical diagnosis. Such a study is being conducted at Rochester, MN.

In addition a careful review of the literature on dementia since 1907 has been done. Special attention has been given to the cases of dementia originally described in Alzheimer's laboratory in Munich (West Germany). Using the United Nations population projections for the 20-year period 1980-2000, the possible effect of demographic trends on senile dementia prevalence in several "developed countries" (United Nations definition) has been studied.

A unique opportunity is available to study the population of the Honolulu Heart Project. This cohort of people have been followed prospectively for over 20 years and are now in the high risk age-group for Alzheimer's disease. These people have been subject to repeated interviews and serum analysis. These prospectively collected data will be studied as risk factors for Alzheimer's disease. In addition, some members of this cohort, who are all of Japanese origin, have come to autopsy. Some studies have suggested that multi-infarct dementia, as a cause of dementia, is more common in the Japanese than Alzheimer's disease. Our study should be able to address this question definitively.

The Branch is also interested in accurately documenting possible racial differentials in the prevalence of major neurologic disorders. A number of early investigations suggested possible differences by race, but were based on hospital or clinic experience and could not identify a well-defined population from which cases were derived. Population-based studies followed, but questions concerning the results centered on possible racial differentials in access to expertise in neurologic diagnosis and treatment. We reinvestigated (in conjunction with the Surveys and Demographic Studies Branch, BFSB, IRP, NINCDS) this problem of possible racial differentials in the prevalence of major neurologic disorders by surveying a well-defined population (approximately 25,000, almost equally divided between blacks and whites). We developed a strategy which eliminated the requirement that persons must have entered the health-care system for detection of disease. The disorders investigated included cerebral palsy, dementia, psychomotor delay, epilepsy, Parkinson's disease, essential tremor, and cerebrovascular disease (both transient ischemic attacks and completed stroke). The basis of the investigation was a door-to-door survey which utilized a detailed questionnaire inquiring not only about diagnoses, but also about signs and symptoms suggestive of neurologic dysfunction. Over 97% of the households agreed to the interview. Those household members suspected of having one of the disorders of interest were then asked to have a neurologic examination conducted by a senior, board-certified neurologist. The interviews and examinations have been completed, and the data are being edited and analyzed. Data currently available for Parkinson's disease indicate that in the population studied, the disorder is more common in whites but the difference between races is not as great as suggested by earlier studies. The same survey yielded information on essential tremor, thereby providing the first data on the prevalence of this condition in a defined U.S. population. For either race, the prevalence ratios were slightly greater in women, and for either sex, the figures were slightly higher for whites. In this same population, it was also possible to measure the prevalence of cerebral palsy. Prevalence ratios of cerebral palsy were higher in males than in females, and greater in blacks than in whites.

Variation in mortality rates by race and sex for the entire U.S. for the years 1971 and 1973 through 1978 were also studied for 20 categories of neurologic diseases. For 14 of the 20 categories of neurologic diseases studied, the average annual age-adjusted mortality rates were higher in males than in females and for 11 categories, the average annual age-adjusted mortality rates were higher in whites than in nonwhites.

Uniform strategies to study neurologic disease have been developed for application in developing countries (e.g., Nigeria, Ecuador, India, the People's Republic of China, Peru, Ecuador, Chile, Tunisia, Senegal, and Venezuela), in collaboration with the World Health Organization. Preliminary results from pilot studies in Nigeria and the People's Republic of China have already revealed interesting findings. For example, migraine is as common among a rural black African population as among urban populations of Western Europe. Furthermore, epilepsy is a major problem in Nigeria, with a prevalence considerably higher than reported in developed countries. In areas of Beijing and Harbin, northern cities of the People's Republic of China, the incidence and prevalence of cerebrovascular disease is higher than anywhere else in the world where this problem has been studied. In addition, stroke follows a definite geographic pattern in China with the lowest rates occurring in southern China. A protocol to study the problem of mental retardation is being developed. This protocol will be applied in Ecuador, India, and the People's Republic of China.

We currently have very little information on the patterns of medical care received by all individuals with neurologic disease in a given community. The Branch is, therefore, studying this problem in Rochester, MN. Although the findings of this investigation will not necessarily be applicable to other regions of the U.S., the city of Rochester does offer particular advantages. Cases of neurologic disease among residents have already been identified through previous studies. Medical encounters are easily documented through a records-linkage resource. In addition, Rochester residents have access to high-quality medical care, and physicians with neurologic expertise are available within the community. Thus, the Rochester experience may provide some estimate of the pattern of medical care in the ideal situation in which the population has ready access to neurologic expertise, and in which there is little financial restraint to such care. The study for patients with brain tumor is being prepared for publication, and similar data are being analyzed for completed stroke.

Although death certificate data are limited by possible misdiagnosis, incomplete case ascertainment, errors in coding, etc., detailed morbidity information on neurologic diseases for the entire U.S. and for other countries is not available. The Branch has analyzed mortality data for selected neurologic disorders by country and by county in the U.S. The overall patterns which emerge may be useful in evaluating trends over time and in formulating etiologic hypotheses. Among the most interesting findings is that the mortality from cerebrovascular disease has decreased in most developed countries over a 20-year period. This trend is not universal, however. For multiple sclerosis, countries initially reporting high mortality rates have generally reported declines, so that more recent mortality data for multiple sclerosis by country show less of a differential than previously reported. U.S. mortality rates for motor neuron disease and anencephaly were analyzed by county. For anencephaly, counties in the Mississippi River region and in the Appalachian Region had the highest rates. With regards to motor neuron disease, counties in the west (especially the northwest) had the highest rates and there was a positive association with rural farming. These leads will be pursued in more definitive studies.

Many neurologic disorders (such as epilepsy) are important causes of morbidity during life and may contribute to mortality indirectly. The potential for neurologic diseases to indirectly lead to death has been studied by analyzing national mortality data for the U.S. for the years 1971 and 1973 through 1978. Marked differences were found in the mortality rates for deaths due to and related to 19 categories of neurologic diseases studied. For example, the mortality rates for deaths related to epilepsy are more than double the rates for deaths due to epilepsy. This suggests that mortality data for epilepsy based on underlying cause considerably underestimates the magnitude of the problem.

Diseases occurring together may provide important information in the search for etiology. Association of diseases occurring at the time of death was also studied for all deaths occurring in the U.S. from 1971, and 1973 through 1978. Case-control studies for associated conditions at the time of death for patients dying due to motor neuron disease, epilepsy, nervous system tumor, and cerebrovascular disease without hypertension have been conducted. Results have provided important new information; for example, the frequent association of infections with motor neuron disease suggests that aggressive management of infections may prolong longevity in these patients.

A number of other collaborative projects include the investigation of space/time clusters of neurologic disease (with the Centers for Disease Control and the Government of Colombia), the development of survey strategies (with the World Health Organization and the Section on Disease Statistics Surveys), a study of myasthenia gravis and multiple sclerosis in the same patient (with the Mayo Clinic), an investigation of neurologic disorders during pregnancy and the postpartum period (with the Mayo Clinic), a study of the epidemiology of eye tumors (with the Connecticut State Department of Health), the effect of weather on the incidence of stroke (with the Mayo Clinic), and international comparisons in the incidence of brain tumors. Finally, extensive reviews have been prepared on the epidemiologic aspects of Huntington's disease, otitis media, Alzheimer's disease, cerebrovascular disease, primary intracranial tumors, Tourette's syndrome, peripheral neuropathy, neurologic diseases in the elderly, controlled therapeutic trials of motor neuron disease, epilepsy, descriptive, analytic, and experimental methods in neuroepidemiology, statistical methods for calculating confidence intervals, and procedures for neuroepidemiologic investigations in developing countries.

The clinical neurogenetics component of the program involves three areas: 1) genetic-epidemiologic studies of movement disorders (e.g., the dystonias); 2) genetic-epidemiologic studies of multifactorial neurologic disorders (e.g., Parkinson's disease, Alzheimer's disease, and multiple sclerosis); and 3) genetic and biochemical studies of hereditary nervous system tumors.

Collaborative studies are underway with personnel in IRP, NINCDS to explain our observations of altered dopamine beta hydroxylase and norepinephrine levels in blood and biopterin in cerebrospinal fluid (CSF) in genetic subsets of dystonia patients. Based on low CSF biopterin in a form of familial dystonia, biopterin has been administered intravenously to 10 patients resulting in transient improvement in several.

Together with colleagues at Johns Hopkins Hospital we have reported the first example of Munchausen Syndrome presenting as torsion dystonia. The patient underwent brain surgery, tracheostomy and feeding gastrostomy before she told us of the factitious basis for her dystonia-like symptoms.

Genetic study of 41 monozygotic twin pairs and 19 dizygotic twin pairs, selected because at least one member had Parkinson's disease, revealed only one monozygotic twin pair and none of the dizygotic pairs definitely concordant for the disease. Although the unaffected co-twin in each case remains at risk, this very low concordance suggests that neither conventional environmental nor genetic factors are critical determinants. Analysis of clinical and psychological observation and interview data on 21 monozygotic twin pairs discordant for Parkinson's disease indicates life-long differences in personality are present in affected versus unaffected twins, as our preliminary study suggested. These observations together with data which indicate a uniform distribution of Parkinson's disease about the world and a stable occurrence over this century have suggested a novel etiologic theory based on initial dopamine neuron number.

An hereditary leukoencephalopathy simulating MS, with onset at about age 35, is under study in two large kindreds. Derangement of the autonomic nervous system is often seen early in the course and when recognized, serves to distinguish this single gene disorder clinically from multiple sclerosis of the chronic progressive type. Computerized tomographic and nuclear magnetic resonance scans are highly characteristic.

Our studies have led to the recognition of at least two distinct genetic forms of neurofibromatosis: 1) the classical form as described by von Recklinghausen, and 2) a form in which bilateral acoustic neuromas are the hallmark. We have focused on neurofibromatosis with bilateral acoustic neuroma. Efforts have been directed at improving and simplifying screening high-risk individuals, confirming diagnosis, and establishing criteria for intervention. Audiologic studies, including evaluation of auditory-evoked response and acoustic reflex decay, are a useful, non-invasive means for early detection of acoustic neuroma and for following their growth.

In our first major study involving neurofibromatosis of the von Recklinghausen type, a multidisciplinary program is in progress to evaluate specific neurologic and cognitive status in patients and their unaffected sibs.

Awards to Branch personnel:

Dr. Schoenberg's contribution to the science of neuroepidemiology was recently recognized by his being awarded the NIH Outstanding Service Medal for consistent contributions to the field of epidemiology as they relate to cerebrovascular disease and its magnitude, distribution, and risk factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 01924-15 NEB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical, Genetic, Pathophysiologic Study of Hereditary Movement Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Roswell Eldridge   Medical Geneticist, NEB, IRP, NINCDS

COOPERATING UNITS (if any)

ET, IRP, NINCDS; HE, NHLBI; LCS, DCBR, NIMH

LAB/BRANCH

Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.75	0.25	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects   ☐ (b) Human tissues   ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In this project, we seek to 1) clarify and expand the nosology of the hereditary movement disorders; 2) contribute to the understanding of the underlying biochemical basis; 3) determine the most effective treatment for each disorder; and 4) suggest guidelines for counseling individuals at risk. General syndromes under study include the dystonias, tic disorders, blepharospasm, and myoclonus. Approaches include standard epidemiologic and clinical genetic studies together with collaborative efforts in evaluating the role of neurotransmitters such as dopamine, their precursors, and metabolites, and their necessary cofactors.

Collaborative studies are underway with personnel in LCS, DCBR, NIMH to explain our earlier observations of altered dopamine beta hydroxylase and norepinephrine levels in blood and biopterin in CSF in a genetic subset of dystonia patients. Members of selected families are being brought to the Clinical Center, NIH, for trial of several new pharmacological agents.

Biopterin administered intravenously has led to acute benefit in one form of generalized dystonia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01927-15 NEB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical, Genetic, Pathophysiologic Study of Hereditary Nervous System Tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Roswell Eldridge Medical Geneticist, NEB, IRP, NINCDS

COOPERATING UNITS (if any) OP, CC: SN, IRP, NINCDS; Division of Medical Genetics, Dept. of Pediatrics, Children's Hospital National Medical Center; Dept. of Neurosurgery, Massachusetts General Hospital, Boston, MA

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Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.00	0.75	0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In this project we seek to define and classify hereditary tumors of the nervous system; to add to the clinical description and natural history of these diseases; to suggest methods for early diagnosis; to evaluate present modes of treatment: and to develop methods for preclinical detection and screening.

Our studies have led to the recognition of at least two distinct genetic forms of neurofibromatosis: 1) the classical form as described by von Recklinghausen, and 2) a form in which bilateral acoustic neuromas are the hallmark. We have focused on neurofibromatosis with bilateral acoustic neuroma. Efforts have been directed at improving and simplifying screening of high-risk individuals, confirming diagnosis and establishing criteria for intervention. Audiologic studies, including evaluation of auditory-evoked response and acoustic reflex decay, are useful means for early documentation and monitoring of acoustic neuroma.

In our first major study involving neurofibromatosis of the von Recklinghausen type, a multidisciplinary program is in progress to evaluate neurologic and cognitive status in these patients compared to their unaffected sibs. Initiation of gene linkage studies, so successful in Huntington disease, awaits availability of modest funds, primarily for travel.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02167-11 NEB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic Epidemiology Studies in MS and Other Multifactorial Neurologic Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Roswell Eldridge Medical Geneticist, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)*

NI, IRP and OBFS, OD, NINCDS; M CN NIMH; Department of Neurology, Monmouth Medical Center, Monmouth, NJ

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Neuroepidemiology Branch, Intramural Research Program

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INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 2.5	PROFESSIONAL: 0.5	OTHER: 2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In this project we are coupling genetic and environmental studies in selected families and twin pairs with disorders such as multiple sclerosis, Parkinson's disease, and Alzheimer's disease, in an effort to distinguish specific contributing factors.

A multi-disciplinary study of 41 monozygotic twin pairs and 19 dizygotic twin pairs, selected on the basis of at least one member being diagnosed as having Parkinson's disease has led to the novel hypothesis that at least some cases are due to a reduced number of critical neurons in the substantia nigra and related structures very early in life.

An hereditary leukoencephalopathy simulating MS with onset at about age 35 is under study in kindred with over 20 affected. Derangement of the autonomic nervous system is often seen early in the course and when recognized, serves to distinguish this single gene disorder from multiple sclerosis clinically. Computerized tomographic scan changes of the brain are dramatic.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02240-09 NEB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Epidemiology of Dementia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bruce S. Schoenberg Chief, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)* Epidemiology, Demography, and Biometry, NIA; W. Massey, M.D., Duke Univ.; E. Kokman, M.D. and J.P. Whisnant, M.D., Mayo Clinic; B. Jordan, Harvard Medical School; M. Alter, Temple Univ.; E. Kahanah, Hadassah Hospital, Jerusalem, Israel; R. Katzman, Albert Einstein College of Medicine, New York

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INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A number of different approaches are being utilized to estimate the mortality and morbidity of Alzheimer's disease/senile dementia in several population groups in the U.S. and to measure the distribution of this disease in segments of the population.

To study international variation in the epidemiology of Alzheimer's disease, a uniform protocol for definition of disease and methodology has been developed. This is now being applied in the U.S. in Denver, Colorado, and White Plains, N.Y., and in a multicenter study in Italy. There have been many requests to apply this protocol in many different parts of the world.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02241-09 NEB

PERIOD COVERED
October 1, 1984 through September 20, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Epidemiology of Cerebrovascular Disease in Adults

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Bruce S. Schoenberg Chief, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)*
J.P. Whisnant, M.D., Mayo Clinic; D.G. Schoenberg, M.S., Bethesda, Maryland, A. Lilienfeld, M.D., Johns Hopkins University

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INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	2.8	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This investigation is aimed 1) at evaluating the effect of heart disease and hypertension as potentially treatable precursors or completed stroke and transient ischemic attacks; 2) at documenting unusual patterns of cerebrovascular disease; 3) at determining the autopsy patterns for patients dying with cerebrovascular disease in defined community; and 4) at examining if weather parameters have any effect on stroke incidence.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02243-09 NEB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pediatric Neuroepidemiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Bruce S. Schoenberg, Chief, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)* D. Schoenberg, M.S., Research Epidemiologist, Bethesda, Maryland; J.F. Mellinger, M.D., M.R. Gomez, M.D., L.T. Kurland, M.D., Dr.,P.H., and R.V. Groover, M.D., Dept. of Neurology, Mayo Clinic; L.L. Salkowicz, P. Gunderson, Ph.D., Minnesota Department of Health

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INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The project documented the frequency of primary intracranial neoplasms in the pediatric populations of Rochester, MN, and the state of Connecticut. In addition, using the records-linkage system available for residents of Rochester, MN, we investigated the magnitude and risk factors for cerebrovascular disease in infants and children.

The same Rochester, MN, records-linkage system was used to determine temporal trends in the incidence rates of cerebral palsy as well as the distribution of clinical subtypes and survival by clinical subtype, for the years 1950-1976. For the state of Minnesota, sex-specific neonatal mortality rates (NMR) in gestational age/birthweight risk subgroups were delineated for the years 1970-1976, and sex- and birthweight-specific NMR trends were determined for the years 1967-1976.

The same record linkage system has been used to identify all possible cases of complex partial seizures occurring in the years 1935-1979. A case-control study is being conducted to identify risk factors associated with the occurrence of such seizures. Similiar studies will be conducted for tonic-clonic seizures, absence seizures and myoclonic seizures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02297-09 NEB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mortality from Neurologic Disorders: National and International Comparisons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bruce S. Schoenberg Chief, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)*

W. Massey, M.D., Duke University; D.G. Schoenberg, M.S., Bethesda, Maryland

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Neuroepidemiology Branch, Intramural Research Program

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NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	6.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Although death certificate data are limited by possible misdiagnosis, incomplete case ascertainment, errors in coding, etc., detailed morbidity information on neurologic diseases for the entire U.S. and for other countries is not available. The Branch has analyzed mortality data for selected neurologic disorders by country and by county in the U.S. The overall patterns which emerge may be useful in evaluating trends over time and in formulating etiologic hypotheses.

Some neurologic disease may contribute to death indirectly. Since there are no uniform criteria for what constitutes the underlying cause of death in patients, it is important to examine all deaths in which a disease is listed as an underlying, immediate, associated or contributory cause of death to get more complete information about the relationship between the disease and death. Mortality data for the U.S. for deaths due to and related to twenty neurologic diseases were studied.

Diseases occurring together may provide important information in the search for etiology of diseases. Association of diseases occurring at the time of death was also studied for all deaths occurring in the U.S. from 1971 and 1973 through 1978.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	**PROJECT NUMBER** Z01 NS 02299-09 NEB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Reviews of Epidemiologic Aspects of Neurologic Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Bruce S. Schoenberg Chief, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)*
W. Massey, M.D., Duke University; D. Schoenberg, M.S., Bethesda, Maryland

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INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Development of new neurologic studies requires thorough historic and methodologic reviews of prior investigations. These yield important unexplored etiologic clues that may be investigated using current technology. Major emphasis has been to cerebrovascular disease, otitis media, inherited ataxias, Huntington's disease, febrile seizures, Tourette's syndrome, peripheral neuropathy, neurologic disease in the elderly, controlled therapeutic trials of motor neuron disease, epilepsy, descriptive, analytic, and experimental methods in neuroepidemiology, statistical methods for calculating confidence intervals, procedures for neuroepidemiologic investigations in developing countries, and epidemiologic studies of Primary Degenerative Dementia.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02300-09 NEB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Course and Medical Care for Neurologic Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bruce S. Schoenberg Chief, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)*

J. P. Whisnant, M.D., Department of Neurology, Mayo Clinic, Rochester, MN

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Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	2.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The study uses a review and abstraction of data from records for a selected group of neurological disorders. It obtains the items of data necessary to determine onset of the disorder, duration, data and cause of death, or current status. These data will be used to construct modified life tables to estimate the expectation of life after diagnosis, the survival curve and morbidity and severity estimates. It will also include analysis of type and duration of medical care received by patients with neurologic disorders derived from a well-defined population.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02301-09 NEB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Collaborative Studies of Less Common or Less Debilitating Neurologic Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Bruce S. Schoenberg Chief, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)*
M. Zack, M.D., Atlanta, Georgia; Neurosciences Program, WHO, Geneva, Switzerland; D. Duane, M.D., B. Sandok, M.D., Mayo Clinic; G. Roman, Bogata, Colombia; P. S. Spencer, Albert Einstein College of Medicine, New York

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Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.5	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A number of collaborative efforts involve the investigation of the characteristics of unusual or less debilitating (e.g., headache) neurologic disease phenomena. Unusual associations or space/time clusters of neurologic disorders may provide leads to etiology or therapy. These may be tested through more formal approaches.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02305-09 NEB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Epidemiology of Intracranial Neoplasms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Bruce S. Schoenberg   Chief, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)* B.W. Christine, M.D., M.P.H., Connecticut State Department of Health; J.P. Whisnant, M.D., and R.J. Campbell, M.D., Mayo Clinic; L. Mahalak, M.D., Jackson, MS; A. Heck, M.D., Univ. of TN; R. Simon, M.D., Berkeley, CA; B. Jordan, B.A., Harvard Medical School

LAB/BRANCH
Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects   ☐ (b) Human tissues   ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Branch has conducted extensive investigations on the descriptive epidemiology of primary intracranial neoplasms using data from population-based registries worldwide. Analytic studies were carried out to investigate the relationship between intracranial neoplasms and tumors occurring at other sites. These studies included careful review of tumor nomenclature, disease definitions, and survey strategies. A case-control study of glioblastoma multiforme is now being conducted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02307-09 NEB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Educational Resources in Neurological Epidemiology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
Bruce S. Schoenberg Chief, NEB, IRP, NINCDS

COOPERATING UNITS (if any)
D. Schoenberg, M.S., Research Epidemiologist, Bethesda, Maryland

LAB/BRANCH
Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Because there is severe shortage of available manpower in neuroepidemiology, the Branch has developed an active teaching program for current and future collaborative investigators. A series of six video tapes produced by the Branch are distributed on a loan basis without charge. A textbook, entitled NEUROLOGICAL EPIDEMIOLOGY: PRINCIPLES AND CLINICAL APPLICATIONS, has been prepared. In cooperation with the World Health Organization and the World Federation of Neurology Research Committee on Neuroepidemiology, formal courses were conducted in Caracas, Venezuela, Shanghai, the People's Republic of China, Nijmegen, the Netherlands, and Bombay, India. Additional courses will be held in Jerusalem, Israel, and Hamburg, West Germany.

A set of video tapes have been produced for training interviewers in the methodology of interviewing for case-control studies. This has been done in both Italian and in English.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02370-07 NEB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

*Racial and Geographic Differences in Occurrence of Neurologic Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Bruce S. Schoenberg Chief, NEB, IRP, NINCDS

COOPERATING UNITS (if any) OBFS, OD, NINCDS; A. Haerer, M.D., Univ. of Mississippi; U.S. Bureau of the Census; C.L. Bolis, M.D., (WHO); B.O. Osuntokun, M.D.(Nigeria); E. Garcia-Pedroza, M.D.(Mexico); Wang Chung-cheng, M.D.(People's Rep. of China); E. Bharucha, M.D.(India); M.C. Gutierrez del Olmo, M.D., & A. Portera-Sanchez, M.D.(Spain); J. Cabrera, M.D.(Peru); P. Ponce, M.D.(Venezuela), & Dr. M. Cruz(Ecuador)

LAB/BRANCH

Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
11.0	8.0	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this study is to accurately document possible racial differentials in the prevalence of major neurologic disorders by surveying an entire county, with a biracial population of approximately 25,000. The disorders investigated include cerebral palsy, dementia, psychomotor delay, epilepsy, Parkinson's disease, essential tremor, and cerebrovascular disease.

Variation in mortality rates by race and sex for the entire U.S. for the years 1971 and 1973 through 1978 were also studied for 20 categories of neurologic diseases.

In addition, research protocols for neuroepidemiologic studies in developing countries have been prepared for Ecuador, Mexico, Nigeria, Peru, the People's Republic of China, Spain, Venezuela and India. Pilot investigations have been successfully carried out in Ecuador, Mexico, Nigeria, Peru, the People's Republic of China, and India.

[Former title: Racial Differentials in the Prevalence of Major Neurologic Disorders and Surveys in Developing Countries].

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02423-06 NEB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of Data Resources for Neuroepidemiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Bruce S. Schoenberg   Chief, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)* F. Clifford Rose, M.B., F.R.C.P., B. Benjamin, Ph.D
S. Haberman, M.A., F.I.A., and R. Capildeo, M.B., B.S., Charing Cross
Neuroepidemiology Unit, London, England; W. Sibley, M.D., Univ. of Arizona,
Tucson, Arizona; E. Kahanah, M.D., Neurology Unit, Ashkelon, Israel; Y. Leibowitz,
Neuroepidemiology Unit, Jerusalem, Israel

LAB/BRANCH
Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects   ☐ (b) Human tissues   ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To develop 1) a registry of hospitalized patients with neurologic disease in a well-defined population of 3.5 million people, and 2) resources for case-control studies of neurologic diseases using uniform methods of data collection.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02424-06 NEB

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Standardized Nomenclature and Coding of Neurologic Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bruce S. Schoenberg Chief, NEB, IRP, NINCDS

COOPERATING UNITS *(if any)* L. Kurland, M.D., Mayo Clinic, Rochester, MN; J.F. Kurtzke, M.D., Georgetown Univ., Washington, D.C.; F. Clifford Rose, M.B., F.R.C.P., B. Benjamin, Ph.D., S. Haberman, M.A., F.I.A., and R. Capildeo, M.B., B.S., Charing Cross Neuroepidemiology Unit, London, England; L. Schut, M.D., Minneapolis, MN; and K. Kondo, M.D., Tokyo, Japan

LAB/BRANCH

Neuroepidemiology Branch, Intramural Research Program

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	2.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To develop an internationally acceptable standard of nomenclature, classification, and coding of neurologic disorders.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02570-03 NEB

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Natural History of ALS-PD in Guam

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Bruce Schoenberg, M.D., Chief, Neuroepidemiology Branch, IRP, NINCDS

COOPERATING UNITS *(if any)*
NONE

LAB/BRANCH
Neuroepidemiology Branch, IRP

SECTION
Guam Research Section

INSTITUTE AND LOCATION
NINCDS, Tamuning, Guam 96911 and NINCDS, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.6	1.6	6.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

As a continuation of previous projects on clinical, pathological, and epidemiologic surveillance of Guamanian amyotrophic lateral sclerosis (ALS) and Parkinsonism-dementia (PD) in the Mariana Islands, a total of 112 cases, including suspects registered as of January 1, 1983, are to be followed at intervals of six months for detailed clinical descriptions of patterns of progression by a qualified neurologist until all of the patients expire. It has been learned that the average duration of ALS is 4.0 to 4.5 years after onset with a range of 2.0 to 25 years. The study of those long surviving cases (over ten years) has been completed. Clinically they showed three patterns: (1) onset with slowly but steady progression at the same pace throughout the course; (2) rapid progression to complete paralysis of the limbs within 1.5 to 3 years and then remaining practically stable for the next 5 to 10 years; (3) onset with minimal atrophy and weakness for the first 5 to 6 years and then rapid step-wise progression to death. A study of the neuropathology of these long-surviving cases by a guest neuropathologist from Japan showed a burnt-out picture: few active areas of neuronal, axonal, or myelin destruction with the remaining neurons appearing surprisingly healthy.

A significant number of PD cases were found to show not only lower motor neuron involvement but also severe pelvicrural flexion contractures in the advanced stage of the disease. This observation presents an important question of: (1) motor neuron involvement as a part of the natural history of chronic diseases of the CNS, or (2) a process identical to ALS which occurs in the same patient. If the latter is true, these cases may represent a continuum of ALS and PD, and thus indicate a single etiology of these two diseases.

TAB 19 -- NEUROIMMUNOLOGY BRANCH -- (NI)

ANNUAL REPORT

October 1, 1984 through September 30, 1985

National Institute of Neurological and Communicative Disorders and Stroke

Neuroimmunology Branch

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Annual Report
October 1, 1984 to September 30, 1985
Neuroimmunology Branch
National Institute of Neurological and
Communicative Disorders and Stroke
Dale E. Mc Farlin, M.D., Chief

Research in the Neuroimmunology Branch (NIB) includes investigations of both fundamental immunological mechanisms and disorders of immune function in neurological diseases. Both experimental diseases and human diseases as well as clinical trials of pharmacological agents and procedures which modify immune reactivity are being studied. A major strength of the NIB research is the complimentary nature of the clinical and basic investigations.

Fundamental research on the mechanisms responsible for T-cell recognition and activation has continued. In recent years, a number of molecules on the surface of human T-cells have been identified. Progress is being made in determining the biological function of such components. The T4 molecule has been shown to be expressed almost exclusively on T-lymphocytes which react with antigen in association with class II major histocompatibility (MHC) molecules. Analysis of $T4^+$ cytotoxic T-lymphocyte (CTL) clones indicates that the T4 molecule is involved in conjugate formation between $T4^+$ CTL and target cells but is not required for antigen recognition by T-cells. The T8 molecule has been shown to be expressed on T-lymphocytes which react with antigen in association with class I MHC molecules. On the basis of these findings, it was proposed that the T4 and T8 molecules act as accessory adhesion structures that react with non-polymorphic epitopes on class II and class I MHC molecules, respectively, and thereby provide an accessory binding function which is required by T-cells with relatively low affinity antigen-specific receptors.

In order to analyze the function of T8 and T4 molecules, as well as other surface components such as LFA-1 and LFA-2, experiments using DNA-mediated gene transfer have been initiated. The human gene that encodes the heavy chain of a class I MHC molecules (HLA-A3) has been isolated and used to tranfect mouse L cells. After establishing that the human gene product had been expressed on the cell surface using specific antibodies, the capacity of human anti-HLA-A3 CTL to recognize and lyse the transfected target was assessed. The preliminary results indicate that functional human CTL recognition of mouse cells transfected with one of the human class I MHC genes can be achieved and that such cells can be effectively lysed. These findings indicate that the only human gene product on the target cell required for recognition by CTL with this specificity is the class I heavy chain. Furthermore, antibody blocking experiments demonstrated that the effector cell molecules T8 and LFA-1, but not LFA-2, are functionally involved in recognition of the transfectants.

The fundamental studies of interactions between sensitized T-lymphocytes and targets have applications to a number of areas including the investigation of immunologically mediated central nervous system (CNS) disease. Experimental allergic encephalomyelitis (EAE) has been extensively studied by the NIB. A highly sensitive and reproducible method for adoptively transferring this disease in mice was developed in the past, and it is the focus of considerable investigation. The transferred disease is characterized pathologically by infiltration of mononuclear cells and the presence of primary demyelinization. Of particular interest is the fact that virtually all of the mice affected by the acute disease recover and subsequently develop a chronic relapsing course. These observations have prompted a number of important questions which can be approached experimentally. For example, what mechanisms are responsible for the initial disease? What are the mechanisms responsible for recovery and subsequent relapses? Because the disease is produced by the transfer of lymphocytes sensitized to myelin basic protein (MBP) and not by the transfer of antigen or antigen-presenting cells, it has been postulated that an early event in the pathogenesis of the first attack involves interaction between sensitized immune cells and the capillary endothelial cells (EC).

Experiments designed to seek an interaction between CNS EC cells and sensitized lymphocytes are currently in progress. Immunocytochemical studies have shown that class II MHC molecules are only minimally expressed on CNS EC in situ and are not detectable on EC freshly isolated from mouse brain. However, culturing freshly isolated EC with preparations containing gamma interferon led to the expression of class II MHC molecules on the cell surface. Functional studies conducted in parallel showed that freshly isolated CNS EC lack the capacity to present MBP to lymph node cells depleted of antigen-presenting cells. In contrast, EC which contain Class II MHC molecules induced with gamma interferon can present antigen. These findings support the concept that an interaction between sensitized immune cells and EC can occur, but indicate that an additional signal leading to the expression of class II MHC molecules on the EC surface membrane is required. The capactiy of factors produced by lymphocytes to induce class II MHC molecules on EC is currently being examined. It is noteworthy that a unique property of CNS EC is that they have tight junctions which form the blood brain barrier. Consequently, the studies of CNS EC have broad implications to the important phenomenon of leukocyte migration into the CNS.

In the relapsing model of EAE produced by adoptive transfer of immune cells, the mechanisms for subsequent relapses are not known. One possibility is that the transferred T-cells both produce the initial attack and give rise to progeny that are also responsible for subsequent episodes of demyelination; the chronic immune response could either occur in lymphoid organs or the CNS. A second possibility is that during the course of the disease sensitization to myelin components other than MBP occurs. Evidence supporting the second possibility has not thus far been obtained and pathological evaluation of animals with chronic relapsing EAE has shown the presence of lymphocyte infiltrates which are often tightly packed with mosaic-like reticulated chambers created by flat processes from surrounding cells. The organization of such CNS infiltrates resembles lymphoid tissue in which interaction among various immune cells occurs. Other investigators have shown that class II MHC molecules can be induced on astrocytes which then acquire the potential to present antigen to immune lymphocytes. The organized inflammatory infiltrates observed in our experiments may represent lymphocyte and astrocyte interactions within the nervous system which maintain the ongoing immune reactivity responsible for the chronic disease.

The fundamental studies of T-lymphocyte function also have significant implications for the cellular immune response (CMI) to measles and other viruses. The Cellular Immunology Section recently demonstrated that MHC-restricted T-cell lysis of measles virus-infected targets is mediated by $T4^+$ CTL which are restricted by class II MHC molecules. Over the past year, research in this area has been expanded and MHC restricted CTL directed at several viruses including influenza and mumps have been identified. Measles virus, however, remains somewhat unique because it primarily evokes CTL which are $T4^+$ and restricted by class II MHC molecules. A number of measles-specific T-lymphocyte clones have been generated. Some of these proliferate in response to the virus; others are CTL, and some clones both proliferate and have cytotoxic activity. The measles-specific T-cell clones are being used to identify the class II MHC components responsible for the restriction. For given clones, either DR or DP (SB) molecules were found to be restriction elements.

Many of the measles-specific class II restricted CTLs were derived from a patient with multiple sclerosis (MS) and are restricted by DR2. It is known that DR2 is supertypic for at least five HLA-D types, defined by mixed lymphocyte reactions. The expression of the DR2 beta two chain has been shown by others to vary in these various HLA-D types. Experiments were conducted to determine if the five HLA-D subtypes can function as effective targets for DR2 restricted measles-specific CTL. All five HLA-D subtypes could be infected with the virus, but only two, HLA-Dw2 and HLA-Dw12, could be recognized by the measles-specific CTL clones derived from the patient with MS. This functional difference correlated with the presence of a specific DR2 beta two chain. These observations established that the delineation of HLA-DR2 into various subgroups has functional significance which is associated with molecular differences within HLA-D region. These results may also have implications for the association between MS and HLA-Dw2.

The antigen specificity of the various measles-specific T-cell clones is also being assessed. Measles virus consists of five structural polypeptides. Sufficient quantities of each of these components for specificity studies have been purified. The findings indicate that all five components can stimulate human T-cells to proliferate and that CTL clones can be generated against the two surface components of the virus, the fusion protein and the hemagglutinin. The Edmonson (Ed) strain of virus has been used in the studies to date. However, the research is being extended to the hampster neurotropic (HNT) strain of measles virus because this strain produces a persistent infection in experimental animals, is neurovirulent, and because there are biochemical and immunochemical differences between the polypeptides of the Ed and HNT strains.

The clinical research in the NIB is closely linked to the fundamental investigations. For example, in assessing the relationship between immune function and MS, considerable emphasis is being placed on the genetic background. These studies include the investigation of twins as well as sporadic patients with well defined genetic makeup. In the past, considerable effort has been devoted to developing specific assays for measuring CTL generated against viruses. These approaches are currently being conducted in these patient populations. The preliminary findings indicate that patients with MS have reduced capacity to generate measles-specific CTL.

The longitudinal comparison of concordance of MS in monozygotic (MZ) twins as compared to dizygotic twins has continued. MZ twins were previously found to have higher concordance which supports the concept of a genetic contribution to pathogenesis. In addition, abnormalities of cerebrospinal fluid (CSF) immunoglobulins indistinguishable from those seen in MS were identified in many clinically normal twins. The evaluation of clinically discordant sets of twins by magnetic resonance imaging (MRI) has continued in collaboration with the Radiology Department. Some individuals with clinically definite MS have normal MRI scans; however, at the opposite extreme has been the identification of white matter lesions by MRI in normal identical twins of individuals with MS. This observation and the CSF findings indicate that subclinical demyelination may be much more common than previously recognized. At the present time, it is not known whether this conclusion applies to the general population or just to first degree relatives of MS patients.

The patient populations which have been well characterized immunologically and genetically provide an excellent resource for clinical trials. Two phase one and a phase three trial in MS patients are currently in progress. The demonstration of immunoregulatory abnormalities in some patients with MS has provided the basis for a trial of lymphocyte transfer in MZ discordant twins. In this protocol blood lymphocytes are removed by lymphocytophoresis from the normal twin and transferred into the affected twin who is evaluated by clinical and immunological parameters. This procedure has been conducted in two MZ twin sets; no changes were observed in one of these. In the second twin set the affected individual had a progressive course associated with increased immunoglobulin synthesis in CSF over the four years before entering the protocol. Since the first transfer of normal lymphocytes approximately two years ago, the clinical course has remained stable and serial CSF studies have shown a normalization of IgG synthesis. These observations suggest that transfer of normal lymphocytes may have modified both the clinical manifestations of the disease, as well as the immunoglobulin abnormalities. This approach will be extended to other MZ discordant twins, if appropriate sets can be identified.

The open trial of Poly ICLC in chronic MS has been continued. This agent is an interferon inducer and immunomodulating agent. Approximately 70 percent of patients in the trial have completed the course of Poly ICLC. Particular attention is being given to evaluation of the clinical course following termination of this experimental treatment. Interferon production and immune function are being examined in each patient. An unexpected finding was that females produced statistically less interferon than males in response to Poly ICLC. This prompted a limited study of interferon production in response to Poly ICLC in monkeys which showed that female monkeys also produced statistically less interferon than male monkeys in response to Poly ICLC. These observations may have implications for the reports of reduced interferon production in MS.

A collaborative phase three trial of cyclosporine A in patients with active progressive MS has been initiated. Patients in the double-blind trial are randomized into groups which receive either cyclosporine A or placebo. Detailed clinical assessments are conducted periodically, and CSF immunoglobulins are quantitated before, during, and at the completion of the treatment period. The NIB patients also will be extensively studied using ancillary tests which may reflect subclinical changes. These include evoked response studies which will be done at six-month intervals and MRI. Immunological function will be evaluated using the specific assays of cell-mediated immunity described above. This will provide longitudinal information about immunological function in patients who are well characterized clinically. The data will be valuable in correlating immunological findings with disease activity. Consequently, the study should provide a better understanding of the disease process, as well as establishing the effectiveness of cyclosporine A treatment.

Over the past three years, the activities of the clinical neurophysiology unit established by Professor Fritz Buchthal have been part of the NIB research program. This group provided service to the Clincial Center and conducted original research on neuromuscular diseases. During the past year, Professor Buchthal left the NIH and these activities were transferred to the Office of the Clinical Director.

The value of immunological approaches for investigation of the nervous system, as well as the relationship between the immune and neurological systems, has been increasingly apparent. Although the NIB has provided assistance and advice to many neuroscientists in the past, a significant portion of our research effort has not been directly involved in these areas. Currently, three new initiatives are under way. Interaction between endorphins and serum antibody in normal individuals and patients with affective disorders is being studied in collaboration with scientists in NIMH. Secondly, the influence of neuropeptides on cellular immune function is being investigated. Thirdly, a new section on immunopharmacology has been established with Dr. Irwin Kopin as a Section Head. The research of this group has been delayed until laboratory space is available. However, it is planned that this section will study interactions between various neurotransmitters and receptors in the nervous system. Focus will be placed on the application of contemporary concepts of immune network to these areas. It is anticipated that these investigations will be extended into human disorders and animal models of neuronal dysfunction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	**PROJECT NUMBER** Z01 NS 02202-10 NI

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunological Studies in Patients with Multiple Sclerosis and other CNS Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Dale E. McFarlin, Chief, Neuroimmunology Branch, NINCDS

COOPERATING UNITS *(if any)*
ID, NINCDS

LAB/BRANCH
Neuroimmunology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.0	4.0	3.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Investigation of patients with Neurological Dysfunction. The general aim of this project is to obtain more precise understanding of multiple factors possibly related either singly or in combination to the pathogenesis of a number of neurological disorders including multiple sclerosis, myasthenia gravis, polyneuropathy and other neuromuscular diseases. The studies of multiple sclerosis include a detailed evaluation of the histocompatibility makeup and the relationship between immunogenetic background and clinical disease as well as immunological function including the cellular response to various human viruses. Magnetic resonance imaging is being used to assess the extent and magnitude of lesions in the white matter. These studies are performed in patients with sporadic disease, patients with a family history of demyelinating disease as well as identical and nonidentical twins who are either concordant or discordant for the disease. Cerebrospinal fluid immunoglobulin content and specificity are being evaluated by new highly sensitive techniques. Trials of experimental therapeutic approaches are being conducted in carefully selected patients with multiple sclerosis. One phase I trial currently in progress involves the administration of Poly ICLC, an interferon inducer. In another phase I trial blood lymphocytes are being transferred from a normal person to an identical twin with multiple sclerosis. A phase III cooperative trial of cyclosporine A in chronic progressive multiple sclerosis has been initiated. Patients in this study are randomized into groups that are treated with either cyclosporine A or placebo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02203-10 NI

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
The Immune Response Against Membrane Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
PI: Dale E. McFarlin, Chief, Neuroimmunology Branch, NINCDS

COOPERATING UNITS (if any)
Dpt. of Biochemistry and Molecular Biology, Harvard Medical School, Boston, MA.
Laboratory of Immunogenics, NIAID, NIH
Virology Department, Karolinska Institute, Stockholm, Sweden

LAB/BRANCH
Neuroimmunology

SECTION
Neurological Diseases Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The major goal of this project is to characterize virus antigens and otner components expressed on the surface of infected cells which are the targets of the immune response. Monoclonal antibodies against the five structural proteins of measles virus have been produced and are used to characterize and purify these viral components. Focus has been placed on the two components, the hemagglutinin (HA) and the fusion (F) protein, expressed on the membrane surface. The HA protein is being studied in the Edmonston and hampster neurotropic strains of measles. Immune cells recognize viral antigen(s) in association with components of major histocompatibility complex (MHC). The relationship between human MHC molecules and measles HA and F proteins is being examined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	**PROJECT NUMBER** Z01 NS 02204-10 NI

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* Immunologic Mechanisms Operative in Experimental Autoimmune Diseases of the Nervous System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Dale E. McFarlin, Chief, Neuroimmunology Branch, NINCDS

COOPERATING UNITS *(if any)*
Departments of Pathology (Neuropathology) and Neuroscience, Albert Einstein College of Medicine, New York, N.Y.

LAB/BRANCH
Neuroimmunology

SECTION
Neurological Diseases Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of this project is to identify the mechanisms responsible for the production of experimental allergic encephalomyelitis, a model of autoimmune disease whicn is manifested by demyelination. This disease is being studied in mice because this species is ideally suited for the analysis of immunologic and genetic factors which lead to disease. Three forms of the murine disease: 1) Acute Experimental Allergic Encephalomyelitis, 2) Chronic Relapsing Experimental Allergic Encephalomyelitis and 3) Adoptively Transferred Experimental Allergic Encephalomyelitis have been produced. Research is currently being focused on the adoptively transferred form of the disease. The transfer of lymphocytes sensitized against myelin basic protein leads to acute neurological dysfunction which is characterized pathologically by inflammation and primary demyelination. Many mice recover and develop chronic relapsing disease. The subpopulation of lymphocytes responsible for the tranferred disease has been identified and the mechanisms related to the migration of immune cells across the blood brain barrier into the nervous system are being assessed. Interactions between immune cells and capillary endothelial cells are being studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02205-10 NI

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Interaction Between Viruses and the Host Immune System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Henry F. McFarland, Deputy Chief, Neuroimmunology Branch, NINCDS

COOPERATING UNITS *(if any)*
LMB, NINCDS
ID, NINCDS

LAB/BRANCH
Neuroimmunology

SECTION
Cellular Immunology Section

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	4.0	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this study is to examine the host immune response to viruses. The major goal is to examine the normal immune response to naturally occurring viruses in man and to extend these studies to patients in order to identify abnormalities of immune regulation which may be related to the pathogenesis of certain diseases of the nervous system. These studies involve a functional analysis of the cellular immune response to measles virus and other viruses of man. This includes studies of cytotoxic, helper and suppressor T- cell populations. The genetic influence on the generation and expression of these responses is being examined. In addition, T-cell lines and clones are also being used to examine cellular reactivity to these viruses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02603-02 NI

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Molecular Mechanisms of Lymphoid Cell-Cell Interactions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
PI: William E. Biddison, Sr. Investigator, Neuroimmunology Branch, NINCDS

COOPERATING UNITS (if any)
Immunology Branch, NCI

LAB/BRANCH
Neuroimmunology

SECTION
Cellular Immunology

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to define the mechanisms by which T-cell surface molecules function in the recognition of foreign cell surface molecules. A large panel of DP-specific cytotoxic T-cell (CTL) clones has been developed to facilitate the study of membrane molecules that are involved in T-cell recognition and triggering. These CTL clones have been used to analyze the roles of the T3, T4, T8, and LFA-1 surface molecules in T-cell recognition of the class II major histocompatibility complex (MHC) antigens. The results indicate that the role of the T4 molecule may be to facilitate T-cell recognition of class II molecules by reacting with a nonpolymorphic region of the molecule and thereby increasing the overall tightness of binding of T-cells to target cells. The T3 molecule appears to be involved in an affinity-dependent triggering function and is not involved in target cell binding. Studies have also been conducted on the molecular requirements for CTL recognition of target cells using the technique of DNA-mediated gene transfer. The gene which encodes the heavy chain of an HLA-A3 molecule has been transfected into murine L cells. The HLA-A3 molecule is expressed at the surface of the transfected cells and these transfectants are susceptible to lysis by HLA-A3-specific CTL. Antibody blocking studies indicate that the T8 and LFA-1 molecules are functionally involved in recognition of the transfected cells. These results demonstrate that the only human gene product on the target cells that is required for HLA-A3-specific CTL recognition is the HLA-A3 heavy chain, and that the target molecules of the putative cell interaction molecules T8 and LFA-1 are either on the HLA class I heavy chain or on murine molecules that are highly homologous to their human counterparts.

TO - NIB/IRP

TAB 20 -- SURGICAL NEUROLOGY BRANCH -- (SN)

ANNUAL REPORT
October 1, 1984 through September 30, 1985
Surgical Neurology Branch
National Institute of Neurological and Communicative Disorders and Stroke
Paul L. Kornblith, M.D., Chief

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ANNUAL REPORT

October 1, 1984 through September 30, 1985
Surgical Neurology Branch, IRP

National Institutes of Neurological and Communicative Disorders and Stroke

I. SUMMARY OF STUDIES

The Surgical Neurology Branch's (SNB) principal activities concern the following sections: 1) Clinical Neurosurgery Section; 2) Tissue Culture Laboratory; 3) Cellular Immunology Unit; 4) Cellular Biology Unit; 5) Biochemistry Unit; and 6) Electron Microscopy Unit.

The SNB has as its major research function the study of the biology and the therapeutic approaches to the problem of the malignant tumors of the brain. Its clinical function is to provide the neurosurgical services to its own research protocol patients and to patients seen in consultation in the NIH Clinical Center. The SNB is presently located in Building 10A, 10 and 9. Its staff includes 10 clinical neurosurgeons at various levels of training and experience as well as 3 senior scientists, 4 junior scientists and a support staff of 20 technical and administrative individuals.

In addition to its primary functions of clinical and basic research the SNB serves as a major resource for young neurosurgeons to experience the excitement of a combined clinical and neuroscience environment. Of the 30 individuals who have participated in the SNB program as medical staff fellows and senior staff fellows, all but the earliest participants have already entered or are planning to enter academic roles.

In the SNB research program, there have been major advances within the past year. These advances have been at both the basic research and clinical levels. It has been found that IL-2 (Interleukin 2), a lymphokine, can stimulate autologous lymphocytes from glioma patients to become specifically cytotoxic to the tumor cells of that patient. This basic laboratory observation has now been extrapolated to the clinical arena and 8 patients have now been treated with these "LAK" cells, administered directly into their tumors.

So far there has been no toxicity and there appear to be potential responders. This work has been carried out by Drs. Elizabeth Grimm and Steven Jacobs. A second area involves the finding that, in tissue culture studies, high concentrations of chemotherapeutic agents have a much wider spectrum of efficacy and thus the use of intraarterial therapy with subsequent dialysis cartridge drug removal pioneered by Dr. Edward Oldfield has shown both experimental and clinical promise in improving chemotherapy for brain tumors. In a rather striking observation, Drs. Kufta and Staunton found that the drug spirohydantoin, a"dilantin mustard," has specific efficacy in brain tumors without the bone marrow suppression characteristic of other agents. Furthermore, this agent causes a confusional state similar to Alzheimer's disease which is completelyreversible with continuous use of intravenous physostigmine. The potential role of differentiation agents has been suggested by in vitro studies which have shown not only tumor cell growth

READEX FICHE

suppression and morphological differentiation but also a decrease in plasminogen activator.

The research program has now been established to work on many of the major basic questions in glioma biology and the clinical program now can provide the type of neurosurgical care needed to address the problems of glioma patients at any stage of disease.

A. CLINICAL NEUROSURGERY SECTION

The clinical activities of the Surgical Neurology Branch are primarily directed to the investigation of the biological behavior and mechanisms of pathophysiology of malignant primary brain tumors, pituitary tumors, acoustic neurinomas, spinal cord AVMs, and the surgical management of epilepsy refractive to medical therapy.

Malignant Primary Brain Tumors

1. INTRAARTERIAL CHEMOTHERAPY (Clinical project Nos. 84-N-41, 84-N-78, 85-N-14)

One of the basic tenets of anticancer chemotherapy is that increased tumor exposure to drug should result in increased tumor response. One method currently being used to increase drug exposure of malignant brain tumors is by intracarotid infusion. Although intracarotid infusion increases drug delivery, systemic toxicity, not brain toxicity, frequently limits the tolerable dose. A means of extracting the drug from the blood after one passage through the brain, and before the high concentration of drug reaches the general circulation, should allow dose escalation to levels which provide increased tumor response. Since last year we have continued attempts to develop techniques which provide regional vascular isolation of a tumor-bearing region by percutaneous catheterization of the afferent and efferent vessels. This permits the venous drainage of the region to be circulated extracorporeally for drug removal before the high concentration of drug reaches the systemic circulation. The following have been demonstrated since last year's report.

a) A pilot study of 4 patients treated with intracarotid BCNU during extracorporeal hemoperfusion of the jugular venous blood demonstrated that the drug exposure of the body could be reduced by 56-87% by channeling the blood extracorporeally at 300 ml/min through a hemoperfusion cartridge for drug extraction. The pharmacokinetic advantage (ratio of brain exposure to body exposure) compared to intravenous infusion of the same dose was 21-55:1.

b) Techniques to deliver intracarotid infusions of BCNU distal to the origin of the ophthalmic artery were evaluated and found successful and have now been shown to eliminate the retinal toxicity and visual loss which frequently occur after intracarotid infusion of BCNU into the portion of the carotid artery proximal to the ophthalmic artery.

c) Drug streaming during intracarotid delivery results in maldistribution of the infused agent with the potential of delivering very high (toxic) concentrations of drug to some regions of the brain, while other areas receive minimal drug. Patients who received intracarotid BCNU were studied with CT scanning, PET scanning, and MRI and the results correlated with histopathology to demonstrate that 1) progressively enlarging cerebral lesions which are often seen on CT and MRI scans following intracarotid chemotherapy may not be tumor recurrence but sites of focal cerebral necrosis, 2) drug streaming is probably the cause of focal encephalomalacia following intracarotid infusion of BCNU.

d) Drug streaming was studied in rhesus monkeys by comparing the distribution of the deposition of ^{14}C-iodoantipyrine during two methods of intracarotid infusion. A rapid retrograde infusion eliminated the prominent heterogeneous distribution of drug deposition which occurred during the slow infusion (the slow infusion was at a rate analogous to that which is currently being generally used clinically). This study strongly suggests that current methods of intraarterial drug delivery, to the brain and other sites, are associated with an unpredictable and variable drug distribution and that this maldistribution can be eliminated by techniques, such as a rapid infusion, which eliminate intraarterial streaming.

e) A dye dilution study of intracarotid infusion of indocyanine green in rhesus monkeys demonstrated that the recovery of substances from the jugular blood after intracarotid infusion is linearly related to the rate of aspiration of the blood from the jugular blood. We have recently demonstrated a similar relationship using indocyanine green during intracarotid drug infusion in humans. This suggests that the amount of an injected drug which can be removed from blood before the blood is returned to the body is dependent on the rate at which blood is withdrawn from the jugular vein. With an effective extracorporeal device for extracting drug, it may be possible to remove as much as >95% of an injected dose before the drug reaches the general circulation.

f) A preliminary study, performed in vitro, demonstrated that about 90% of the cisplatin in whole blood circulating at 300 ml/min could be removed by hemodialysis using 2 hemodialysis cartridges in series. We then treated 4 patients with cisplatin by intracarotid infusion of a dose that is widely used intravenously. Extracorporeal circulation of the jugular blood for drug removal during intracarotid infusion resulted in tumor exposures 5-15 fold greater than the exposure of the remainder for the body. We have now performed 3 treatments in humans using a very high dose of cisplatin (200 mg/m^2) combined with drug removal by extracorporeal hemodialysis. The results of the systemic drug levels suggest that the body was exposed to less than 1/2 of the exposure expected if the drug-removal technique had not been used. It is too early to evaluate tumor response, but there has been no apparent brain injury from the high-dose intracarotid infusion.

The above studies were performed in collaboration with Drs. Robert Dedrick of the Bioengineering and Instrumentation Branch of the Division of Research Services and Dr. John Doppman of the Diagnostic Radiology Department, The Clinical Center.

II. ELABORATION OF A FACTOR BY MALIGNANT GLIOMAS WHICH INCREASES THE PERMEABILITY OF NORMAL BLOOD VESSELS IN VIVO

One of the pathophysiological mechanisms of the production of an intracranial mass effect by primary and secondary malignant tumors is by the tumor eliciting cerebral edema in the surrounding normal tissue. We have demonstrated that media from malignant gliomas in monolayer cultures secrete a substance which, when injected intradermally into guinea pigs, increases the accumulation of a circulating radioisotope (I^{125}RISA) and a marker dye (methylene blue) at the site of injection compared to media from fibroblasts, benign brain tumors, normal saline and tissue culture media. The production of the increased vascular permeability factor in the media from malignant gliomas can be abrogated by incubation of the tumor cells with dexamethasone and by inhibition of protein production by cyclophosphamide. Preliminary work toward isolation of the factor suggests that it is a 45,000-60,000 molecular weight protein.

III. PET SCANNING WHILE USING BARBITURATE ANESTHESIA TO ACCENTUATE THE DIFFERENCE IN GLUCOSE METABOLISM OF MALIGNANT GLIOMAS AND NORMAL BRAIN (CLINICAL PROTOCOL 85-N-14)

This project involves FDG-PET scanning of patients who have brain tumors before and during deep barbiturate general anesthesia. The results indicate that a profound reduction of cerebral glycolytic activity can be achieved with a level of anesthesia which produces burst-suppression EEG activity. Gliomas, however, have only a minimal change in glycolytic rate under the barbiturate anesthesia. The results also indicate that lower grade lesions, which are not visible on PET scans performed with the patient awake, become visible as background synaptic activity is suppressed with barbiturates. The true extent of growth into the surrounding tissue by higher grade lesions can be better appreciated when background activity is reduced under the barbiturate anesthesia. This work provides evidence that barbiturates may allow a "reverse contrast enhancement" of lesions with decreased neuronal activity. This phenomenon may provide basis for development of specific antitumor therapy. The technique also may be valuable in studying other pathological processes such as degenerative diseases, epilepsy, movement disorders, cerebral infarction and head injury.

IV. SYSTEMIC CHEMOTHERAPY

1. Spiromustine Phase I Study

A Phase I disease-specific trial of spiromustine (spirohydantoin mustard), a drug synthesized to cross the blood-brain barrier and function as a tumoricidal agent, was initiated in early 1984 in patients with malignant gliomas. Twenty-two patients have now entered into the study at escalating doses.

The dose-limiting toxicity in all patients was a neurologic syndrome of acute confusion, hallucinations, dry mouth and fixed, dilated pupils. This occurred to some extent in all patients treated at 6.6 mg/m^2 or higher doses, and was shown to be due to an anticholinergic effect. This was rapidly reversed with physostigmine.

One tumor completely remitted on CT scan. A number of patients exhibited partial remission by CT associated with clinical improvement or stabilization of previously progressive disease.

Pharmacokinetic study demonstrated a serum half-life of approximately 15 minutes. An analysis of the emulsion in which the drug was given to patients showed rapid drug decay. From this we determined that the drug must be prepared immediately before administration. Patient's peak serum levels were shown to increase significantly when this methodology was followed.

This study suggests that spiromustine has activity against malignant gliomas in vivo and that the dose-limiting toxicity is an acute anticholinergic syndrome, reversed (at least in part) in all patients with physostigmine.

The maximal totalled dose has been established in the Phase I trial. A Phase II study is in preparation. It is designed to assess the efficacy of spiromustine in patients with malignant glioma when given at a standard dose.

2. AZQ Trial in Malignant Glioma

The Phase II trial of AZQ in patients with malignant glioma which opened 3 years ago was closed this year. Fifty-three evaluable patients were accrued and treated with 20 mg/m^2 of AZQ on days one and eight of a twenty-eight day cycle.

The results were: 8/53 patients (15%) improved by CT and/or clinical criteria; 25/53 (47%) stabilized rapidly progressing disease; and in 19/53 (36%) disease progressed despite therapy. A number of patients who improved or stabilized have maintained this response and continue to be followed in the clinic.

There were no drug related deaths. The dose-limiting toxicity was myelosuppression which was cumulative in most patients.

This study demonstrated that AZQ is an effective agent against malignant glioma in a significant percentage of patients, including many of those heavily pre-treated with other forms of chemotherapy. The information obtained has been
pre-treated with other forms of chemotherapy. The information obtained has been
followed by Phase III trials which are ongoing in this country and in Europe.

V. MALIGNANT GLIOMA

Although average survival in patients with malignant glioma is very short once the diagnosis is made, a number of individuals die rapidly while others live many times the average survival, despite uniform treatment. Histopathologic analysis at the time of diagnosis does not allow prediction of the unit of progress of the disease in individual patients.

Positron Emission Tomography (PET) with fluorine-1-2 deoxy-glucose was performed in 45 patients with the diagnosis of malignant glioma with the PET data being correlated with patient survival. A low glucose utilization was associated with a survival 4 times that of patients whose tumor exhibited a high glucose utilization. Therefore PET-FDG scans reflect the biological behavior of malignant gliomas and can be used to predict the survival time of patients who have these tumors.

VI. QUANTITATIVE AUTORADIOGRAPHY WAS USED TO INVESTIGATE SEVERAL ASPECTS OF THE METABOLISM AND THERAPY OF BRAIN TUMOR MODELS IN ANIMALS (Don Wright)

Since glucose metabolism has been demonstrated to be higher in malignant brain tumors than in the surrounding brain tissue and 2-deoxy-D-glucose metabolism is blocked after initial phosphorylation intracellularly, it may be possible to use 2-deoxy-D-glucose as a tumoricidal agent. The following studies were designed to investigate this possibility.

Pharmacokinetics

A two part study of the kinetics of tracer amounts (verifying prior studies) and pharmacologic doses of intravenous and intraperitoneal 2-deoxy-D-glucose was performed in normal rats. The study demonstrated differing blood and tissue time courses of tracer vs. pharmacologic doses of 2DG. Toxicologic (LD_{50}) data included blood and tissue levels in surviving and dying animals. A related experiment evaluated the effect of repeated pharmacologic doses and various schedules to find a combination of dose and interval which would minimize toxicity.

Following these preliminary studies a series of rats bearing subcutaneous and intracerebral Walker-256 tumors were "treated" with pharmacologic doses of 2DG. The half lives for the drug in tumor were 2.5 - 3 times that of normal tissue. An examination of the "lumped constant" for 2DG in pathologic tissue was carried out using a quantitative method (autoradiography) and drug schedules were designed for future treatment studies to minimize toxicity.

1. 2-Deoxyglucose as an Adjunct to Radiation Therapy of Tumors

2-deoxy-glucose was effective in killing subcutaneous tumors in rats. Its mechanism of action makes it an attractive treatment for tumors in synergy to optimize treatment of subcutaneous Walker 256 tumors in rats. A pilot series of rats bearing subcutaneous W-256 were given a five day course of (pharmacologic doses) 2DG combined with five different doses of irradiation delivered on days 2-4 of the 2DG "therapy". Additive effects of 2DG + XRT were demonstrated (measured by tumor volumes). Future studies are planned to assess the effects on an intracerebral model.

2. DMSO Blood-Brain Barrier Effects (D. Wright, R. Blasberg)

An intraarterial model was developed in rats for production of reversible blood-brain-barrier disruption.

a) Dose-Response/Toxicology. The toxicity (target organ) and neurologic effects of intracarotid DMSO (3 dosages, 3 infusion schedules) was established. An optimal dose and infusion schedule was determined (40% DMSO; 0.45 cc/min x 3.3 min).

b) Transfer Constants following blood-brain-barrier disruption. (Influx/efflux, K_1/k_2). AIB (an amino acid analog and a frequently used marker for assessment of blood-brain-barrier permeability) and quantitative autoradiography was performed in rats over 4 time points (3.3 min to 24 hours) to determine the degree and reversibility of BBB disruption using the dose/schedule developed above. BBB disruption was generally reversed within 2 hours.

^{125}Albumin, and ^{51}Chromium-red blood cells were infused in DMSO treated animals to analyze any changes in the volume of the vascular space and extracellular space, as well as to correct the AIB data. DMSO was found to minimally affect these parameters.

3. Effect of Dexamethasone on Cerebral Edema Following Freeze Injury

A freeze injury model that we had previously developed was used to investigate steroid effects on capillary permeability and extracellular space volumes. ^{125}Albumin was infused following production of a cortical freeze injury in rats. A control/steroid pretreatment group comparison showed a decrease in the volume of the extracellular space in the dexamethasone group.

4. Gallium-EDTA Positron Imaging

Tumor bearing dogs were studied with ^{68}gallium-EDTA positron scanning to investigate the integrity of the blood-brain-barrier of this tumor model. Quantitative analysis of influx and efflux constants, and vascular and extracellular spaces was performed. A series of tumor bearing dogs were also imaged using dynamic PET scans. The data was analyzed, using a novel method developed by C. Patlak for transfer constants and vascular spaces. (This work was done in collaboration with Ron Blasburg in the Nuclear Medicine Department)

VII. Additional Laboratory Projects

Brain Tumor Protein Chemistry

We continue our efforts to study proteins associated with human brain tumors using two-dimensional gel electrophoresis, silver staining, electroimmunoblotting and co-migration techniques. Protein patterns associated with a wide variety of both benign and malignant brain tumors have been established, and about 12 major proteins seen on the 2D maps have been identified. This completes the first phase of these studies.

Intermediate filaments (IF) are cytoplasmic structures whose characteristic diameter of 10-nm is between that of actin filaments (6-nm) and microtubules (25-nm). Five major subclasses of IFs are described -

tonofilaments, desmin, vimentin, glial fibrillary acidic protein (GFAP) and neurofilaments (NF). Using two-dimensional gel electrophoresis (2DE) and silver staining, we have previously characterized the protein patterns associated with various human brain tumors. Electroimmunoblotting has now enabled us to identify three major IF proteins on these gels: vimentin (found primarily in fibroblasts and mesenchymal cells), GFAP (astrocytes) and the 70 kD NF protein (neurons). Vimentin forms a characteristic cloping complex at the acid end of the gel (molecular weight 57-40 kD; pI 5.3-4.9). GFAP forms a similar, albeit less focused complex (M.W. 49-36 kD; pI 5.7-5.1). The 70 kD NF protein is seen as a small acidic spot (pI 4.9).

Studies were performed in a wide variety of fresh benign and malignant human brain tumors including astrocytomas, ependymomas, medulloblastomas, sarcomas, meningiomas, schwannomas, choroid plexus papillomas, craniopharyngiomas, hemangioblastomas and pituitary adenomas. In addition to being present in normal cerebral cortex, vimentin was detected in varying amounts in every tumor type studied. Particularly large amounts of this protein were found in meningiomas and hemangioblastomas. As expected, GFAP was present in high concentrations in tumors of glial origin, but was also noted in medulloblastomas, schwannomas, meningiomas, hemangioblastomas and choroid plexus papillomas. The 70 kD NF protein was found only in normal cerebral cortex and in high grade astrocytomas.

During cellular differentiation IF proteins assume new distributions and have been linked to a variety of regulatory functions. Their functional significance in vivo is currently being actively investigated by several groups. In view of the widespread distribution of IF proteins in human brain tumors, these studies could yield valuable insights into tumor biology.

Pituitary Tumors

I. VENOUS SAMPLING TO ESTABLISH THE DIAGNOSIS AND LOCATION OF HORMONE-SECRETING PITUITARY MICROADENOMAS

We have now performed bilateral and simultaneous inferior petrosal sinus sampling in 55 patients with Cushing's syndrome. The results are used, 1) to confirm the diagnosis of patients preoperatively and, 2) to determine the half of the pituitary gland in which a microadenoma resides. The study has been particularly rewarding and has demonstrated the following: 1) sampling from a single inferior petrosal sinus, as has previously been general practice, to establish the diagnosis of Cushing's disease, may be misleading and could result in an incorrect assumption of the source of excess ACTH secretion in as many as half the patients with Cushing's disease, 2) preoperative sampling for ACTH concentrations in the inferior petrosal sinuses determines the site of ACTH-secreting microadenomas within the pituitary gland. Therefore, the surgeon's search for small microadenomas can be focused to one side of the gland which should be helpful in finding smaller tumors. If no tumor is found, the half of the gland containing the microadenoma can be removed. We have no treatment failures in the 30 previously untreated patients with Cushing's disease who have undergone preoperative sampling. The technique has also been used to locate the site of one prolactin-secreting and one TSH-secreting microadenoma preoperatively. This technique, which we recently introduced here at the NIH, is now being widely employed in the evaluation of patients with Cushing's syndrome.

Surgical Treatment of Medically Untreatable Epilepsy

The aim of the surgical arm of the NINCDS epilepsy program is to develop surgical methods for more precise localization and safer resection of epileptogenic foci.

Several surgical procedures and methodologies have been introduced during the past year in the ongoing attempt to better select and treat surgically patients with medically intractable epilepsy. Specially designed and targeted sphenoidal electrodes are now being implanted to help localize epileptogenic foci which originate in the medial temporal lobe and whose electrical projection to the lateral temporal surface can lead to false localization of the source of epileptogenic activity.

Subdural surface electrodes which were designed by the Bioengineering group at NIH, are being implanted in selected patients to record closer to the cortical surface for longer periods than is possible with electrocorticography and to allow direct stimulation of cortical foci to discriminate motor, sensory, and language areas. Such localization helps the topographical identification of overlap between critical cortical areas and epileptic foci.

During surgery for focal epilepsy, depth electrodes are used to record from deep structures inaccessible by routine electrocorticography to identify areas of potential epileptic activity suggested and localized by the new preoperative diagnostic methods, PET scanning, MRI and electromagnetoencephalography.

B. TISSUE CULTURE LABORATORY

The main areas of study for the Tissue Culture Laboratory this past year have included continuation of the pre-screen in vitro microcytotoxicity testing of individual patient tumor lines, characterization of established glioma cell lines, in vitro examination of combination chemotherapy, and a drug screening program for identification of glioma toxic compounds.

Through utilization of an in vitro chemotherapy sensitivity assay developed by Dr. Kornblith, the prospective in vitro response of patient tumor cells to drugs has continued. Over 100 tumor specimens were forwarded to the laboratory for growth and microtiter testing. Besides the specimens obtained at surgery here at the NIH, other cooperative centers included Walter Reed Army Medical Center, George Washington University, Georgetown University, Philadelphia Children's Hospital, Children's Hospital of Washington D.C., Emory Clinic in Atlanta, Georgia, and a number of other centers from across the country.

The anticancer agents aziridinylbenzoquinone (AZQ), cis-platinum and bischloroethylnitrosourea (BCNU) are used in the microcytotoxicity testing for determination of individual patient tumor cell sensitivity or resistance. The results are reported to the referring physician for possible follow-up chemotherapy of the patient. Whereby, the in vitro-in vivo correlation for a clinical predictive value of this testing are most reliable for cells resistant to certain chemotherapeutic agents, in vitro results demonstrating a lack of cytotoxic response to an agent are stressed and subsequent therapy with either a demonstrated sensitive drug or an alternative agent are recommended.

Collaborative studies with Drs. Kurt Kohn and Len Erickson of the National Cancer Institute have shown the importance of individualized glioma patient chemotherapy based on in vitro results. They felt the basis of resistance to BCNU in glial tumor cells was due to the ability of the tumor cell to repair initial DNA damage resulting from drug-induced strand breaks and subsequent interstrand cross-links. Results obtained from use of their Alkaline Elution in vitro DNA assay corresponded with those from the microtiter assay used in our laboratory. Gliomatous cells demonstrating a large quantity of DNA interstrand cross-links also showed significant response to that drug in the cytotoxic test and when further evaluated clinically in a retrospective manner with the patient being subsequently treated with BCNU, his clinical response, as determined by tumor growth via computer tomographic scan, appeared to be positive with no growth being observed. Tumor proliferation was seen in tumors demonstrating a non-significant microcytotoxicity and low interstrand DNA cross-linking phenomena while the amounts of DNA strand breaks exhibited no correlation with either our assay or clinical patient therapy response. The importance of this data is such that we now have multiple in vitro methods of determining sensitivity for resistance of patient tumor cells to chemotherapeutic modalities prior to initiation of therapy and may be able to better select more specific antitumor agents for individualized patient treatments.

Other studies dealing with chemotherapeutic agents involve drug combination treatments and a new drug screening program. Human glioma cell lines once established as monolayers in culture are readily available for a variety of studies. Based on different antitumor mechanisms of action of the agents, it may be possible to combine one or more agents to provide greater cytotoxic effect. Combinations of the antitumor compounds, AZQ, BCNU, and cis-platinum were applied to cell lines in concentrations achievable in plasma and exposed to the lines for varying times. The demonstrated targets of action appear to be as follows: BCNU, AZQ and cis-platinum all have primary alkylating or DNA strand breaking effects. Secondary effects of BCNU involve the cell membrane, AZQ has mitochondrial effects, and cis-platinum affects the cell cytoskeleton. Use of these secondary effects may provide greater tumor cell kill and are the basis of combination therapy. Studies of the combinations of these 3 agents in achievable plasma concentrations are on-going, however, preliminary results of both 4 and 72 hour exposure reveal that AZQ is more cytotoxic alone than in combination with BCNU or cis-platinum. The combination of AZQ and cis-platinum did not prove as cytotoxic as either agent used alone and in fact, demonstrated a cell count similar to control after 72 hours of treatment, suggesting the 2 agents may be negating each's antitumor activity and possibly should not be used together. The cis-platinum and BCNU proved more cytotoxic than cis-platinum used alone. Knowledge of the interactions of different acting compounds is essential for their correct and most efficient use in patient chemotherapy.

Unfortunately, these three agents have a similar dose-limiting toxicity of blood dyscrasias and probably would not be used in combination through conventional systemic treatments. Studies on-going in Dr. Edward Oldfield's Section of Clinical Neurosurgery have shown that much larger doses of cis-platinum and BCNU may be given to patients intraarterially via the carotid artery and removed through dialysis cartridge filtration thereby reducing systemic exposure and blood side effects. Combinations utilizing this approach or a mixture of administrative procedures may enhance the tumor-killing ability of available agents and the efficiency of overall treatment.

An in vitro pre-screen involving a 24 hour microcytotoxicity assay and both 4 and 144 hour growth studies of cultured glioma cells treated with promising new antiglioma compounds provided by the DuPont Company and Arizona State University are on-going. Agents provided by the DuPont Company include both the traditional alkylation chemotherapeutic agent types as well as the newer biological growth control types or "differentiation" agents. Examples of chemicals being screened against our established brain tumor cell lines include retinoids, alkyllysophospholipids, polar compounds, alkylating agents, and numerous butyrates. Antitumor agent data obtained from our screening is compared to that obtained from DuPont animal and xenograft studies utilizing the same agents. Of the eight compounds screened, two appear to have significant cytotoxic effects against malignant brain tumor cells grown in culture. Studies aimed at whether or not certain glial cell markers (S-100,GFAP) and malignancy determinants (plasminogen activator) are affected by these agents are presently being carried out.

Dr. G.R. Pettit, Director of the Cancer Research Institute of Arizona State University, has been one of the leaders in a search for naturally occurring substances possessing cytotoxic brain tumor activity. Agents presently being screened in the SNB Tissue Culture Laboratory have been evaluated against the P388 lymphocytic leukemia. An evaluation of cytotoxic effects through our brain tumor lines could lead to an agent of benefit in the ultimate therapy of our patients. These agents include phyllanthostatins, combretastatins, pancratistatins and the bryostatins, all of which are at various NCI preclinical development stages.

Characterization of the human glioma cell cultures grown from tissue samples is of major importance for further studies utilizing these cells. There are a number of morphological, cell biological and biochemical parameters which are relevant to the characterization of glial tumor cells. Of prime importance to studies of these lines is the identification of cell origin. The antigen that is most useful for this determination is glial fibrillary acidic protein (GFAP). Neurochemical and immunohistochemical studies indicate that GFAP is the sub-unit of glial filaments and is found mostly in neuroglia cells with the only exceptions being the oligodendrocytes and Schwann cells. In addition to determining the source of the tumor cells, the GFAP content of the cells may be directly related to the degree of differentiation of the tumor. The well-differentiated more benign astrocytomas tend to stain strongly for GFAP and contain numerous positive cells while highly malignant astrocytomas or glioblastomas contain few positive cells. Use of this characteristic of the cells can be utilized when evaluating new agents for "differentiating" capability; possibly reverting a malignant cell to a more benign state through drug treatment.

We have screened nineteen of our cultured lines for the presence of GFA protein. Five of these proved positive both by the preliminary pre-screen of indirect immunofluorescence and by a more exact procedure of intermediary filament preparation with 2-dimensional electrophoresis cells and silver staining of slabs for the GFAP marker.

Another protein which we have routinely screened for is S-100. This is a water-soluble antigen which was the first nervous system protein discovered and is so named because of its solubility in 100% ammonium sulfate. It is thought to be generally central nervous system specific and, as such, is not as exact a marker of cell origin as GFAP for brain tumors. Seventeen cultured brain tumors have been screened for S-100 utilizing a technique of solid phase radioimmunoassay developed by Drs. Alan Hirschfeld, Yoshio Moriya and Joseph Bressler. Six of the lines proved positive for S-100 and coincided with the five positively stained GFAP lines. The one line not GFAP (+) but S-100 (+) was an oligodendroglioma cultured line and helped confirm the specificity of the GFAP assay for glial cells.

Other parameters being pursued for characterization of the cultured gliomas include morphologic feature examination both via routine phase and by electron microscopy, growth variability flow cytometric measurements of cellular DNA and karyotyping. In addition a number of human glioma cultured lines have been sent to the DuPont Company for use in collaboration on new antitumor drug screens. They are growing selected malignant lines provided by us in arrhythmic mice resulting in tumors which can then be utilized to test in vivo response to new tumorcidal or differentiating agents having shown effects in the in vitro setting with the same cells used to initiate the animal tumors. The fact that the cells are tumor producing is another facet of the line malignancy and characterization.

C. CELLULAR IMMUNOLOGY UNIT

The Cellular Immunology Unit which has been established during this year under the direction of Dr. Elizabeth Ann Grimm, is dedicated to the study of cytotoxic lymphocytes. The immediate goal of this laboratory is the development and application of means to use cytotoxic lymphocytes for selective lysis of human tumor cells, especially those of the brain. Studies are now in progress in rat and human tumor systems and are being pursued at the molecular, cellular, and in vivo levels. Dr. Grimm has identified an in vitro method to generate tumor selective cytotoxic lymphocytes by activation with the lymphokine, interleukin-2. These lymphokine activated killer cells (Lak) provide a system that obviates the need for specific antigen recognition that has plagued the previous studies by others, including Muul and that of Gately.

The first studies were to determine whether lymphocytes from glioma patients would respond to the IL-2 and create Lak. Dr. Steven Jacobs has been successful in creating these Lak and has found that they will kill both autologous (4/5 tests) and allogeneic (8/8) glioma cells in vitro utilizing a chromium^{51} microcytotoxicity assay. He also has reported that the lymphocytes cultured without IL-2 did not kill the autologous tumor (0/13) nor other tumors, indicating that the lytic activity is induced by IL-2. Normal cells were not lysed. Further experiments were designed to determine the nature of the epitope on glioma cells which render them susceptible to Lak killing. Treatment of the target cells with trypsin (0.1 or 0.01 mg/ml) did eliminate the ability of them to serve as targets for Lak. In contrast, a variety of enzymes and chemicals that alter cell surface carbohydrates had no effect. These results indicate that the moiety on glioma cells which is responsible for their susceptibility to killing by Lak is dependent on a protein determinant.

Because glioma patients receive Decadron^R, which is believed to be a potent immunosuppressive agent, we studied the effect of hydrocortisone (decadron analog) in parallel with another agent, cyclosporine, to determine what effect these drugs had on the activation of Lak. We found that Lak activation was very sensitive to hydrocortisone (10^{-5}-10^{-6}M) and resistant to cyclosporine (10ng-1ug/ml). Allospecific cytotoxic T lymphocytes (CTL) were prepared in parallel and their activation was found to be sensitive to cyclosporine and not to hydrocortisone. Although these results have led us to make further hypothesis concerning the mechanism of Lak activation, they were troubling because of the high levels of Decadron^R received by the glioma patients. We therefore tested patient PBL, from those receiving up to 10 ng/day of Decadron^R. However, it was found that patients receiving even the highest levels of Decadron^R were able to make Lak cells. A second concern was that the Decadron^R perhaps rendered the tumor cells of these patients resistant to killing by lymphocytes. Therefore, it was tested whether this drug affected the susceptibility of cultured glioma tumor to Lak lysis. Glioma cells were treated with hydrocortisone and were tested in parallel with untreated glioma cells as targets. Both were lysed equally well, indicating that the hydrocortisone does not affect the lysis of glioma by Lak cells.

As part of our studies on the activation of Lak cells we know that culture of human peripheral blood lymphocytes in purified natural or recombinant interleukin-2 in the absence of exogenous antigen or mitogen causes the differentiation of nonlytic lymphocyte precursor cells into Lak. We have found that inhibitors of both proliferation and differentiation (gamma irradiation or mitomycin C) do prevent Lak activity. Further studies have been performed to elucidate the mechanism by which IL-2 alone induces the proliferation and differentiation into killer lymphocytes. We have found that the lysosomatropic agents NH_4Cl, chloroquine, or monensin, when preincubated with PBL will prevent the Lak activation, indicating a role for the lysosomes in IL-2 processing. It has been found by us that no IL-2 receptor molecule, defined by the monoclonal antibody Tac--(generously supplied by Dr. Thomas Waldman, NCI) is apparent on Lak precursors. Therefore, we have proposed that a nonreceptor mediated interaction of IL-2 with the cell is obligatory. Tac does appear on these cells after 24 hours in culture when they then need more IL-2 to proliferate. In collaboration with Dr. Anne Walter and Dr. Robert Blumenthal of the Lab. of Mathematical Biology (LMMB), NCI, we have undertaken a study of the means by which IL-2 might interact directly with the lipid bilayer of the lymphocyte cell membrane. Computer generated models of the IL-2 tertiary structure indicate that the IL-2 could aggregate into an ion permeable channel. These results have been conclusive in liposome models and we are now testing them with the lymphocytes.

As part of our studies to define alternative means for Lak activation, we have discovered that the Streptococcus pyogenes preparation OK432, would activate PBL into Lak-like killer cells. (OK432 has been used successfully in

Japan in immunotherapy of intrapleural and ascites tumors, and is currently considered a standard treatment.) The OK432 induced cytotoxic lymphocytes exhibited several properties identical to Lak cells. These included sensitivity of activation to irradiation or mitomycin C, dependence on IL-2, and relative resistance of the killer activity to leucine methyl ester. Masato Yagita is pursuing the description of these cells in parallel with that of Lak and CTL.

Recently our unit has obtained a molecular biologist, Peter Brayton, who is initiating molecular studies of Lak activation. We have performed one experiment in which we look for rearrangement of the T cell receptor beta gene elements and have found that they did not rearrange. To pursue these molecular studies in a controlled manner, Lak hybridomas have been prepared by fusing human Lak cells with a murine thymoma which is HAT sensitive. The fused products have then been cloned, grown into large quantities and are now being tested for Lak activity.

The study of the specificity of Lak lysis is continuing. Not only can we eliminate tumor cell susceptibility to Lak by proteases, but we have been able to create Lak sensitive targets from the normally resistant PBL. This has been performed by modifying proteins on the normal PBL cell surface with a hapten called trinitrophenyl (TNP). Not only are TNP-PBL lysed by Lak but cold target inhibition studies indicated that lysis is inhibited by fresh tumor cells (7/7 experiments) and that tumor lysis is inhibited by TNP-PBL (5/7 experiments). Additionally, it was found that allogeneic tumors totally inhibited lysis of autologous tumors in other cold target studies (3/3 experiments). These results demonstrate the lytic activity expressed by Lak is not HLA restricted, is not limited to tumor cells, and is nonspecific as indicated by the cross reactive recognition of multiple target cell types.

Because of the efficiency and apparent tumor selectivity of Lak lysis, we have proposed a clinical trial of direct intraoperative intracerebral injection of Lak cells in glioma patients. Prior to the initiation of these phase I clinical studies it was essential to determine whether or not Lak cells lysed normal brain tissue. Therefore, we adopted the rat glioma model, using the 9L tumor. Spleen cells from Fisher rats were cultured (10^6/ml) with or without IL-2 and then tested for their ability to lyse the syngeneic 9L glioma cells in vitro in using the standard 4 hour chromium release method, identical to that used in the human. We found that the IL-2 culture did generate rat Lak that would lyse the tumor but not comparably prepared normal rat brain tissue. As in the human, rat lymphocytes cultured in the absence of IL-2 did not generate any cytotoxicity. These results indicate that Lak cells lyse glioma tissue but not normal brain.

The fate of Lak cells following injection into the brain was also pursued in the rat system. Lak labelled with indium 111 were injected into the brain of normal rats. The animals were then sacrificed and the brain removed, sectioned, and autoradiography performed. Our results found that Lak remained localized to the injection site for as long as 72 hours later.

Therefore, a clinical trial (protocol #84-N-238) was initiated entitled, "Immunotherapy of brain tumors by interleukin 2 and interleukin 2 activated autologous lymphocytes." We are injecting in increasing doses either Lak (10^8,10^9 or 10^{10}) cells or IL-2 (10^4,10^5,10^6 units) into the brain tissue surrounding the cavity left following debulking of tumor. To date, six patients have been treated and no obvious signs of toxicity have been observed. These patients have received up to 10^6 units of IL-2 and up to 1×10^8 Lak cells.

D. BIOCHEMISTRY LABORATORY

Monoclonal Antibody Mediated Killing of Tumor Cells

The Unit of Biochemistry, headed by Dr. Richard Youle, is studying the use of monoclonal antibodies to kill disease-causing cells. Monoclonal antibodies which selectively bind tumor cells can be generated, but alone are usually not cytotoxic to the tumor. Toxic proteins such as ricin and diphtheria toxin can be chemically linked to monoclonal antibodies and the new hybrid molecules will bind tumor cells via the antibody moiety and then kill the cells via the toxin moiety. The toxins used are enzymes that catalytically inactivate protein synthesis in target cells with only one or two molecules in the cytoplasm killing a cell. However, the nontarget cell toxicity of the toxins must be blocked with excess ligand to prevent toxin binding and this currently limits this approach to in vitro applications. The cell-type-specific reagents have immediate clinical application in vitro in bone marrow transplantation where T cell depletion improves allogeneic transplantation. The Unit of Biochemistry is supplying these reagents for clinical trials in bone marrow transplantation at the University of Minnesota. Twenty-four patients have now been treated with immunotoxin purged bone marrow as the sole prophylaxis for graft-versus-host disease. Comparing the outcomes with historic controls treated post-transplant with methotrexate, several conclusions can be drawn. The patients had a milder course as evidenced by a significantly shorter hospitalization. Engraftment of donor marrow occurred with a shorter time until leucocyte generation and no severe graft-versus-host disease was seen. Clinical trials of immunotoxin treatment of bone marrow are continuing to increase the patient population and thus the statistical significance of the apparent benefits.

The major goal of the laboratory is to develop immunotoxins which will selectively kill tumor cells in vivo. Currently the limiting step for antibody-toxin hybrids is the entry of the toxin molecule into the cell. Thousands of molecules must bind the cell surface for one molecule to enter the cytoplasm. This low entry rate limits the log kill of these reagents in vitro and explains their frequent failure to eliminate all tumor cells in vivo.

Several new approaches have been successfully applied in vitro and in vivo in the past year. The ricin B subunit was found to increase the specific killing rate of ricin A chain immunotoxins up to 7 fold. A 7 fold increase in rate would increase a 1 log kill to 7 logs, often enough to eliminate the last tumor cell. Unfortunately the B chain was not effective in vivo since it binds 10^7 sites per cell on all the cells in the body and therefore does not reach the tumor cells. We have used several approaches to block the binding site of the toxin B chain while maintaining the activity that increases specific immunotoxin killing.

A) In vitro we previously showed lactose would block nontarget cell killing of intact ricin immuntoxins allowing only a potent antibody mediated toxicity. This has now been successfully applied *in vitro* for human bone marrow transplantation but *in vivo*, the lactose is cleared from the blood within minutes and does not protect animals from ricin B chain mediated toxicity of intact ricin immunotoxins. We have identified a substitute for lactose, asialofetuin, a glycoprotein known to bind ricin B chain which is retained in the vascular system longer than lactose. We found asialofetuin, unlike lactose, will protect mice from ricin toxicity. *In vitro* asialofetuin blocks nontarget cell toxicity of immunotoxins but not target cell toxicity just as lactose does. We have begun *in vivo* therapy of leukemia in animals using asialofetuin to block the nontarget cell killing of intact ricin immunotoxins. Preliminary studies of cancer in animals show a very significant 2-3 fold extension of survival time.

B) We have raised a panel of monoclonal antibodies to study the various activities of ricin. One antibody binds the lactose binding site on ricin and affects immunotoxins *in vitro* like lactose. *In vivo* the MoAb protects mice from ricin toxicity like asialofetuin. Monoclonal antibodies stay in circulation for days and should be even better than asialofetuin for blockage of ricin B chain. We are initiating *in vivo* therapy of cancer in animal models using intact ricin immunotoxins plus the anti B chain monoclonal antibody against the ricin binding site.

C) We have identified the region of the ricin polypeptide chain which binds lactose. Using synthetic peptides identical to various regions in the ricin B chain sequence and injecting these peptides linked to carrier proteins into rabbits we have raised polyclonal antibodies specific for discrete regions on the ricin molecule. Rabbit antibody against a peptide identical to ricin amino acid sequence 60-79, binds ricin and blocks ricin toxicity. Asialofetuin blocks the rabbit polyclonal antibody binding. The rabbit polyclonal antibody blocks binding of the monoclonal antibody. Identification of the sequence of the ricin galactose binding site will help us block the site on intact ricin immunotoxins. We now are chemically modifying amino acids within this sequence and studying their effects on bioactivity. Acetylation of tyrosine residues blocks ricin binding and blocks the monoclonal antibody binding to the ricin galactose binding site. Other amino acids are under investigation. Ultimately, detailed understanding of the structure and function of ricin will allow modifications to be made at the gene level of cloned ricin for ultimate optimization of immunotoxin activity. Our identification of the galactose binding site already tells us what sequence of cloned ricin to delete to decrease nontarget cell toxicity.

D) Toxins may be best adapted for tumor specific toxicity by alterations of amino acid sequence at the gene level. Previously studies were described to understand how to modify the toxin sequence to achieve desired results. Ricin, though cloned in several laboratories, is a complicated eucaryotic protein and has not been expressed in E. coli. Therefore, to begin improving immunotoxins at the gene level we have worked with the prokaryotic toxin, diphtheria toxin. Intact diphtheria toxin was linked to a monoclonal antibody specific for human T cells and was found to specifically kill target cells at 10^{-12}M. The rate of specific killing was 10 fold faster than previously reported immunotoxins. This model system was then used to study cloned fragments of diphtheria toxin. In collaboration with Dr. Larry Greenfield, who has cloned diphtheria toxin (DT), we deleted the C-terminal 15KD region of the toxin which had previously been shown to include the cell surface binding site. This left the toxin A subunit plus a 17 KD fragment of DT B chain thought to facilitate transmembrane transport. When linked to monocloncal antibodies, this truncated DT was 100 fold more toxic than DTA chain linked to antibody and the toxicity was blocked by excess antibody. The cloned DT fragment was 1000 times less toxic to guinea pigs than the native toxin.

Therefore, the fragment of DT B chain included by cloning increased target cell toxicity more than nontarget cell toxicity indicating that separation of B chain entry functions from binding was accomplished to some degree. Comparing intact DT linked to monoclonal antibody with the cloned fragment of DT showed the C-terminal fragment of DT further increased antibody mediated toxicity 100 fold. Therefore, we are now going back to modifications at the gene level in attempts to include the 100 fold activation by the C terminus of DT while omitting regions causing nontarget cell killing.

Mechanism of Toxin Entry into Cells

Toxins like ricin and diphtheria toxin bind the cell surface, are endocytosed, then cross the membrane surrounding the endocytotic vesicle to reach the cytosol where they inactivate protein synthesis. The rate limiting step in toxin and immunotoxin activity is transport across the membrane to the cytosol. How and where this transport step occurs is unknown. To investigate this question we have used hybridoma cells which secrete monoclonal antibodies that block ricin toxicity. We have found that these cells are themselves resistant to ricin because of the antibody they synthesize. We found that the resistance was not caused by extracellular or cell surface antibody but by antibody within the cell in route to secretion. This means that ricin must pass through the protein secretory pathway, comprising the endoplasmic reticulum, golgi apparatus, and secretory vesicles, before entering the cytosol. This new approach used here may be applied to study other toxins, hormones and macromolecules which enter cells by receptor mediated endocytosis.

E. CELLULAR BIOLOGY LABORATORY

In our last report we described our work involving the effects of tumor promoting phorbol esters (PE) on glial differentiation. These compounds have a marked effect on the expression of an oligodendroglial specific property namely, the ability of glucocorticoids to increase glycerol phosphate dehydrogenase (GPDH) levels. Pharmacological evidence, utilizing various PE derivatives, suggested that the effect was mediated by the PE receptor. We found that non-phorbol ester tumor promoter, mezerin (MEZ), and a retinoic acid derivative of a PE, phorbol 12-retinoic 13-acetate (PRA) were more effective than any of the PE in inhibiting GPDH induction. These two compounds are weak stage 1 promoters but strong stage 2 promoters. They bind to the phorbol ester receptor with the binding constant that is less than what is observed for phorbol myristate acetate (PMA), which is the most potent promoter and ligand.

Why are stage 2 promoters more potent than stage one promoters in inhibiting GPDH induction? In glial cells these compounds may have a lower affinity constant for the PE receptor than PMA. This would contradict previous work utilizing brain homogenates which demonstrated that stage 1 promoters have a lower affinity constant for the phorbol ester receptor than stage 2 promoters. The rate of degradation of stage 2 promoters may be slower than the rate for PMA. Therefore the stage 2 promoters last longer in culture. Finally, there may be a minor receptor population which binds stage 2 promoters more strongly than stage 1 promoters and which is responsible for mediating the inhibition of GPDH induction.

Using [20-^{3}H]phorbol 12,13-dibutyrate to characterize phorbol ester binding we found that [^{3}H]PDBU bound to intact cells with an apparent binding affinity of 43±5.2 nM with 4.7+0.5 pmol of [3H]PDBU bound per mg protein. In competition studies using PMA, MEZ and PRA, the apparent Ki values for all three compounds were between 50 and 60 nM. Therefore MEZ, and PRA were equal but not better ligands than PMA for the phorbol ester receptor. Though these binding studies could not fully explain the higher potency of stage 2 promoters in inhibiting GPDH induction, they were surprising since in other tissue stage 2 promoters have a higher Ki than stage 1 promoters. The Ki for PMA agreed with the ED^{50} values for inhibiting GPDH induction, but the ED50 for mezerin and PRA, 5 and 11 nM, respectively, are an order to magnitude lower than the Ki. Thus suggesting a different receptor mediating the event.

The degradation of PMA and MEZ under the same conditions as GPDH induction was also examined. After a two day incubation with the C6 cells, less than 1% of the added [^{3}H]PMA comigrated with the standard PMA, while approximately 60-90% of the added MEZ was degraded under the same conditions. Therefore, mezerin lasts longer in cultures of glial cells.

The slower rate of mezerin degradation may contribute to its higher potency. On the other hand, the discrepancy between the Ki of mezerin and its ED50 for inhibiting GPDH induction suggested that a second receptor may be involved. The major phorbol ester receptor has recently been shown to be the protein kinase C. The natural ligand for this enzyme are the diacylglycerols. We hypothesize that a second class of receptor may have a different endogenous ligand. Therefore we examined whether elevated diacylglycerol levels in C6 cells turn off GPDH induction.

Various drugs have been used to increase endogenous diacylglycerol levels. These include specific ligands, synthetic diacylglycerols, and endogenous choline dependent phospholipase C. We were unable to see any inhibition of GPDH induction when carbachol (which binds to the muscarinic receptor), or 1-oleoyl-2-acetyl-glycerol (a synthetic diacylglycerol) were used. A marked inhibition was observed when the phospholipase C was used. We found that this enzyme induced the release of choline and increased endogenous diacylglycerol levels. We also found that the affinity of [^{3}H]PDBU for its receptor increased form 40+5 nM to 120±11 nM with no change in total number of binding sites. The enzyme lost its activity when boiled for two minutes. Phospholipase A and D did not exhibit activity. This data shows us that ineffectiveness of carbachol and OAG may be due to the amount of time drugs have to be present to see modulations in GPDH activity. At least 24 hours are necessary to see a two-fold increase in GPDH activity. In other systems which have utilized these drugs, no more than a 12 hour incubation time is needed for a increase in their respective biological activity. Therefore, OAG and carbachol may be degraded fast and do not exert an inhibition.

Early work from our laboratory suggested that phorbol esters (and diacylglycerols) may inhibit GPDH induction by lowering endogeous cAMP levels. Other investigators have shown that drugs which increase cAMP levels augment the amount of GPDH synthesized after glucocorticoid stimulation in logarithmic C6 cultures, but have no effect on confluent cultures. We have shown that PMA will completely inhibit GPDH induction in log phase cells, but will only inhibit GPDH induction 60% if the cells are grown in stationary phase. This may be due to the higher sensitivity log phase cells have to cAMP inducing drugs. Therefore the effect of diacylglycerols on beta-adrenergic challenge was explored. Phospholipase C inhibited isoproterenol and forskolin stimulation of cAMP. OAG, phospholipase A and D had no effect. The enzyme had no direct effect on beta receptor binding. Its action may reside on the adenylate cyclase or the GRP binding protein.

Differentiating Glioma Cells

The other approach our laboratory has used in differentiation is to devise an *in vitro* system where glioma cells can be induced to express differentiated properties. For the past year we have concentrated our efforts on one aspect of glial differentiation, the expression of intermediary filaments. In the developing rat, glial cells first express the intermediary filament vimentin. In the adult, vimentin is a characteristic of mesenchyme tissue. During development astroglial cells stop expressing vimentin and begin to express glial fibrous acidic protein (GFAP).

We are now able to quantitate the amount of vimentin and GFAP synthesized by cultured cells by first prelabelling cells with a 35S-methionine, isolate the cytoskeleton due to its insolubility in a nonionic detergent, separate the cytoskeleton components by two dimensional electrophoresis, and then quantitate the amount of radioactivity in the spots which correspond to vimentin and GFAP. We have found that all glioma lines tested express vimentin, and a few coexpress GFAP and vimentin. Also, we and others observed that primary astroglial cultures coexpress vimentin and GFAP.

We have found that compounds with reported differentiating capability, such as retinoic acid, phorbol esters, butyric acid, dimethylsulfoxide, and theophylline have no effect on vimentin or GFAP synthesis in the U-251 glioma cell line. Cells in logarithmic phase express less GFAP than cells in stationary phase. There was no change in vimentin synthesis.

As stated before, some type of cells that normally do not synthesize vimentin will synthesize it when grown in culture. This suggests that culture conditions induce vimentin expression. We hypothesized that two factors involved in *in vitro* growth may be important, the presence of fetal bovine serum and/or attachment to plastic. Therefore the U-251 cell line was grown in the presence of a chemically defined media, or as reaggregating culture, or grown on polylysine or collagen. We found no change in GFAP or vimentin synthesis under any of these conditions. Something was either wrong with our hypothesis or the cell line did not behave appropriately. We repeated some of these experiments with primary astroglial cultures. Monolayer cultures attached to plastic expressed vimentin and GFAP but reaggregating cultures expressed only GFAP. Therefore regulatory mechanisms involved in intermediary filaments (IF) expression may be altered in transformed cells.

Another aspect of glial differentiation being studied is the regulation of intermediary filaments expression. During development astroglial derived cells switch from synthesizing vimentin to synthesizing glial fibrous acidic protein (GFAP). Glioma cells in culture consistently express vimentin and in a few cases coexpress vimentin with GFAP. We have not been able to alter the expression of filament in glioma cells by incubation with differentiating agents. In addition, human glioma cell cultures synthesize vimentin and GFAP when grown as a reaggregating culture, or grown on polylysine or collagen. Primary monolayer cultures of astroglial cells synthesize vimentin and GFAP but synthesize only GFAP when grown as reaggregating cultures. The differences in vimentin synthesis between reaggregating astroglial cell cultures and reaggregating human glioma cultures may reflect changes in regulatory mechanisms which occurred during transformation.

Proposed Course of Study

The discrepancies we have found between the Ki and the ED 50 of mezerin suggests a second receptor. On the other hand, the phospholiphase C data and the degradation data suggest that the protein kinase C is involved and the higher potency of mezerin is due its slower rate of degradation. To resolve the problem of degradation we will measure the amount of GDPH mRNA synthesized after glucocorticoid stimulation and/or MEZ or PMA inhibition. Using a cDNA probe for the GPDH gene, a twenty-fold increase in GPDH mRNA can be measured three hours after glucocorticoid stimulation of C6 cells. This short time in culture minimizes the importance of PMA and mezerin degradation. The cDNA probe for the GPDH gene has recently been made available to our laboratory.

We will also investigate the type of proteins which are phosphorylated after mezerin and PMA stimulation. Ligand stimulated phosphorylation in whole cells is usually complete within 30 minutes, again minimizing the importance of PMA and mezerin degradation. These phosphorylation studies might also help us understand how PMA dedifferentiates cells and induces neoplastic-related properties. PMA has been shown to increase plasminogen activator in glial cells. We have shown that PMA inhibits the expression of one differentiated property. Are both activities mediated by the same mechanism? This can be ascertained by examining the phosphorylation profile induced by PMA and testing agents which augment or inhibit phosphorylation. These same agents will be tested for their ability to inhibit the effect PMA has on GPDH induction and plasminogen activator. Our laboratory has already tested several drugs for their ability to alter the effect PMA has on GPDH induction. Another method which we will use to determine a relationship between phosphorylated proteins and glial function is to use mutant cell lines which lack these proteins and then test these mutants for PMA responsiveness.

We will also localize the site where phospholipase C induces inhibition of cAMP production. The effect of phospholipase C on adenylate cyclase, or the G protein will be measured in both cells and in a cell-free system. We feel that this area is relevant since other investigators have shown that protein kinase C may augment cAMP production in lymphoid cells and pinealocytes.

The relationship between IF expression and neoplasia will also be explored. Presently, evidence suggests that nontransformed cells stop synthesizing vimentin when they grow in reaggregating culture, while neoplastic cells retain the ability to produce vimentin. The source of nontransformed cells used in these studies, primary rat astroglial cultures are not a good control for the human glioma cells. The best comparisons would be to have paired transformed and nontransformed cells lines from the species at similar passages. Unfortunately, we are not aware of an established nontransformed glial cell line which coexpresses vimentin and GFAP from any species. On the other hand, we have begun a collaborative project with Dr. Eugene Major, Infectious Diseases Branch, NINCDS, to characterize some GFAP positive cell lines they have established which are believed to be nontumorigenic. We are presently testing their tumorigenicity in nude mice and their ability to synthesize vimentin during reaggregate and monolayer growth. We are also trying to establish rat astroglial cell lines which are GFAP positive. We will test these cell lines for the ability to synthesize vimentin and GFAP under various growth conditions.

The relationship between IF expression and neoplasia may reside at the genome. Do nontransformed cells contain a regulatory mechanism controlling vimentin synthesis which is lacking in tumorigenic cells? If our initial observations are confirmed we will explore this question by determining the regulatory mechanisms at the translational and transcriptional level. To examine this question, we have obtained the cDNA probe for vimentin. We will also examine a possible role of oncogenes in this expression.

Chemotherapy

We have tested the cytotoxicity of spirohydantoin (shm) mustard in tissue cultures, as reflected by the inhibition of 3H-thymidine uptake in treated cells. A technique has been evolved whereby, shm, which is unstable in aqueous media, can give reliable and reproducible dose response curves in different cell lines. Because of clinical and in vitro evidence of an effect of shm on choline uptake mechanisms, we have examined the cytotoxicity on clones with differing choline uptake and demonstrated preferential toxicity with those of high choline uptake. Similarly we have shown that the toxicity the cell when exposed to shm; again, suggesting that shm may be preferentially taken into cells by an active choline uptake mechanism. At present we are attempting to examine the sensitivity of different cell lines, neuroectodermal and others, to shm with their potential rate of choline uptake, in order to be able to predict *in vitro* sensitivity; and thus possibly to extrapolate this to increases in *in vivo* sensitivity of tumors with enhanced cholinergic activity.

In vivo work on rat brain synaptosomal preparations has shown a 30 to 50% reduction of the high affinity choline transport mechanism, particularly in basal ganglia. Similarly, choline acetyltransferase activity and also muscarinic cholinergic binding sites were reduced. We are presently further characterizing the specificity of this action of shm: tissue levels of succinate dehydrogenase are not altered, but other transmitter systems (e.g. adrenergic) are being investigated.

F. ELECTRON MICROSCOPY LABORATORY

This past year the Electron Microscopy laboratory studied several areas including the conclusion of the comparison of the cellular actions of three antitumor drugs, the study of a fourth chemotherapeutic agent produced by the National Cancer Institute, the morphologic effects of three differentiating compounds on several of our tumor cell lines, computer assisted morphometrics of mitochondria previously treated with an antitumor agent, and provided electron microscopic support for Dr. David Katz, NINCDS neuropathologist.

BCNU, AZQ (aziridinylbenzoquinone) and cis-platinum have been shown biochemically to achieve their antitumor effects by alkylation and/or cross linking of DNA. With transmission electron microscopy, we have studied the in vitro effects of these agents on four glioma-derived cell lines and have shown, through time studies, there is in addition significant non-nuclear cytotoxicity that is important in understanding the action of these agents. BCNU produces prominent membrane surface blebbing that occurs within minutes and is secondary to suppression of intracellular peroxidase activity. For AZQ, mitochondrial toxicity occurs prior to the RNA alkylating effect. Cis-platinum shows a third pattern of cytotoxic damage including dilated subplasmalemmal endoplasmic reticulum and swelling and vesiculation of perinuclear golgi as well as nuclear chromatin clumping consistent with DNA damage.

In collaboration with Dr. James Ellis, Biomedical Engineering and Instrumentation Branch (BEIB), mitochondrial swelling produced by AZQ was studied by computer assisted morphometrics using polygonal approximation. There was a greater variation of size and shape of mitochondria treated with AZQ than with untreated cells. This variation correlated with increases in drug concentration and increased times of exposure. Although other chemotherapeutic agents examined by this laboratory cause mitochondrial damage as cell death occurs, only AZQ causes mitochondrial swelling. This finding strongly suggests a cytoplasmic effect of AZQ involving cellular energetics distinct from its known DNA alkylating effect.

Spiromustine is an antitumor agent produced by the National Cancer Institute which is currently being studied in Phase I clinical trials as well as in in vitro testing. Within cells, it has been shown biochemically to produce DNA alkylation. The cellular effects of spiromustine have been studied by this laboratory with transmission electron microscopy. Four tumor cell lines were used including a high grade and a low grade glioma both of which are known to be resistant to BCNU, a high grade glioma sensitive to BCNU and a sarcoma. In all of the lines examined, after 15-24 hours of exposure to high doses (100-1000ug/ml) of spiromustine, nuclear chromatin clumping consistent with DNA alkylation occurs. The chromatin is clumped throughout the nucleus presenting a different pattern than that previously seen with AZQ or cis-platinum where clumping occurred only along the nuclear envelope. The effect of this drug seems to be strictly nuclear. No distinct cytoplasmic toxicity was observed as was seen by the other chemotherapeutic agent studied. All non-nuclear damage seen related to cell death.

Cellular differentiation can occur in a variety of ways. We have concentrated on chemically induced differentiation of the tumor cells line by cyclic AMP, N, N-dimethylformamide (DMF) and a differentiating agent produced by the DuPont Company.

Three human glioma derived cell lines treated with cyclic AMP and a polar solvent DMF, were examined by transmission electron microscopy. With cAMP the cellular shape changes from bipolar to stellate forms with thin processes of varying number and length. DMF treated cells had fewer processes than the cAMP treated ones. The processes were broader and fewer in number. With both agents cellular organelles increased suggesting greater synthetic and secretory activities indicative of a more differentiated state.

Preliminary studies with the differentiating agent produced by the DuPont company suggest similar changes. Two tumor cell lines are currently being examined. Cells are exposed to varying concentrations of this agent for 24 and 72 hours. Changes in shape and alignment of the cytoskeleton occurs within the first 24 hours. With prolonged exposure and high concentrations of this agent (over 500 ug/ml) cell death occurs.

The DuPont Company is growing selected malignant cell lines, provided by the tissue culture laboratory of this Branch, in athymic mice resulting in tumors to be used for in vivo drug testing. Two of these tumors are currently being examined by our Taboratory using both transmission and scanning electron microscopy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02687-01 SN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Mechanism of Interleukin-2 Activation of Cytotoxic Lymphocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Elizabeth A. Grimm, Ph.D.	Principal Investigator
Peter Brayton, Ph.D.	Senior Staff Fellow
M. Yagita	Fogarty Fellow
Debbie J. Wilson	Technician
Barbara Ikejiri	Technician

COOPERATING UNITS (if any)

LAB/BRANCH
Surgical Neurology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NINCDS, National Institutes of Health, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Activation of cytotoxic lymphocytes is a complex and poorly understood process. Two immunosuppressive drugs, Cyclosporin A (CsA) and Hydrocortisone (Hy) were examined in parallel for their effect on the generation of cytolytic lymphocytes. Peripheral blood lymphocytes (PBL) were stimulated with allogeneic cells to produce allospecific CTL, or with purifed recombinant IL-2 (R IL-2) to activate lymphokine-activated killer cells (LAK). Both CTL and LAK activity were measured in a 4-hour chromium release assay after 7 days of activation. Lysis by CTL was tested against stimulator PBL (not blasts) and LAK against fresh sarcoma tumor cells. At pharmacologic doses, CsA inhibited only CTL generation, while Hy inhibited only LAK. LAK activation is believed to occur by interaction of IL-2 with the precursor cell, via a non receptor mediated interaction. We are attempting to define the exact means of interaction, and then pursue the early events leading to the expression of cytotoxic activity.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02686-01 SN

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunotherapy of Brain Tumors by Interleukin-2 and Activated Lymphocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Elizabeth A. Grimm, Ph.D.	Principal Investigator
B. Holcomb	Student Volunteer
Paul Kornblith, M.D.	Chief, Surgical Neurology Branch
Steven Jacobs, M.D.	Senior Staff Fellow
Debbie Wilson	Technician
Gilbert Melin	Student Volunteer
Catherine Parham	Student Volunteer

COOPERATING UNITS *(if any)*

LAB/BRANCH

Surgical Neurology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINCDS, National Institutes of Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Culture of brain tumor patient peripheral blood lymphocytes (PBL) with recombinant interleukin-2 (IL-2) results in the activation of lymphokine activated killer cells (LAK) with the capacity to lyse autologous and allogeneic glioblastoma. PBL obtained from brain tumor patients were cultured with or without IL-2 for three to seven days and then tested for their ability to lyse target cells in a 4-hour chromium release cytotoxicity assay. PBL were drawn one to two weeks following operative tumor debulking. Cells used as targets included fresh brain tumor cells obtained at the time of craniotomy, fresh brain tumor grown from one to three weeks in tissue culture, fresh autologous PBL and allogeneic glioblastoma grown in tissue culture.

Brain tumor patient PBL cultured without IL-2 did not significantly lyse autologous or allogeneic glioblastoma. However, when these PBL were cultured with IL-2, LAK was generated which produced marked lysis of autologous as well as allogeneic tissue culture glioblastoma in all of eight cases. Significant lysis of autologous fresh tumor by patient LAK was observed in four of five experiments. By contrast, patients' LAK did not kill autologous normal PBL. The ability to generate LAK was not influenced by patient age, previous therapy or the administration of steroids.

Eight patients have received either IL-2 or LAK cells into their brain tissue at the time of surgical debulking of confirmed glioma. No toxicities have been observed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	**PROJECT NUMBER** Z01 NS 02685 01 SN

PERIOD COVERED
October 1, 1984 Through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of Specificity of LAK-Mediated Target Cell Destruction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Elizabeth A. Grimm, Ph.D.	Principal Investigator
Steven K. Jacobs	Senior Staff Fellow
Barbara Ikjiri	Technician
Gilbert Melin	Student Volunteer
William Loundon	Student Volunteer

COOPERATING UNITS *(if any)*

LAB/BRANCH
Surgical Neurology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NINCDS, National Institutes of Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.75	1.75	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Culture of human peripheral blood lymphocytes (PBL) in purified natural or recombinant interleukin-2 in the absence of exogenous antigen or mitogen causes the differentiation of nonlytic precursor cells into lymphokine-activated killers (LAK). A titration of purified Jurkat IL-2 (BRMP, FCRC, NIH) IL-2 showed that the relatively low concentration of 5 U/ml was optimal for LAK activation. The spectrum of target cells susceptible to LAK lysis in a 4-hour cheomium-51 release assay includes fresh NK-resistant tumor cells and trinitrophenyl (TNPO modified autologous PBL. Unmodified PBL are not lysed. Cold target inhibition studies indicated that LAK lysis of autologous TNP-PBL is totally inhibited by fresh tumor cells, and that tumor lysis is inhibited by TNP-PBL. Additionally, allogeneic tumors totally inhibit lysis of autologous tumor cells in other cold target studies. These results demonstrate that the lytic activity expressed by LAK is not HLA restricted, is not limited to tumor cells, and is "polyspecific" as indicated by the cross-reactive recognition of multiple target cell types in these cold target inhibition studies.

The mechanism by which LAK effector cells mediate tumor cell destruction is unknown. Lysis occurs rapidly at 37°, and 4 hours of incubation is optimal. The mechanism is neither an antibody-mediated cytotoxicity (ADCC) nor merely lectin-dependent cytotoxicity (LDCC). The report of successful adoptive therapy in mouse systems with LAK provides the basis for proposing that LAK is a biologically relevent system in which to further examine the mechanism and specificity of cell-mediated cytotoxicity.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02673-01 SN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Monoclonal Antibodies Linked to Ricin for Use in Human Bone Marrow Transplantation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard Youle, Ph.D.	Senior Investigator
Joe Dalton	Chem. Lab Technician

COOPERATING UNITS *(if any)*
Department of Laboratory Medicine, University of Minnesota
Immunology Branch, DCBD, NCI
Laboratory of Mol. Branch (LMB), NIMH

LAB/BRANCH
Surgical Neurology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NINCDS, National Institutes of Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Bone marrow transplantation is the treatment of choice for high risk leukemia, aplastic anemia and other immunodeficiency disorders. It is also being used for therapy of other radiation sensitive tumors and for inherited enzyme deficiency disorders. The limiting complication is graft-versus-host disease (GVHD) caused by mature T cells in the donor marrow recognizing histocompatibility differences between donor and host. Studies in animals and humans have shown that removal of mature T cells from the donor marrow while preserving the pluripotent stem cells prevents GVHD.

Monoclonal antibodies linked to toxic proteins can specifically kill cells based on cell surface antigen differences. We have developed a panel of T cell selective toxins which kill up to 5 logs of T cells at concentrations non-toxic to human stem cells.

We have begun clinical trials of these reagents for 1) prevention of GVHD in MHC matched bone marrow recipents; 2) prevention of GVHD in MHC mismatched BMT. Twenty three patients have now been treated. Ten patients transplanted for high-risk leukemia with major HLA matched sibling marrow are now evaluable. The antibody-ricin conjugate showed no toxicity to the patients. Comparing antibody-toxin treatment with conventional post-transplant methotrexate GVHD prophylactic the hospitalization stay was significantly shorter. Engraftment of donor marrow occurred in all patients and 8 out of 10 showed predominantly donor lymphoid cells 30 days post-transplant. No cases of severe GVHD were observed. Continued clinical trials are underway to increase population size to statistically significant levels to compare GVHD incidence of immunotoxin vs. conventional protocols.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02674 01 SN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Monoclonal Antibody-Toxin Conjugates for Tumor Therapy In Vivo

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard Youle, Ph.D.	Senior Investigator
Marco Colombatti, M.D.	Guest Researcher

COOPERATING UNITS *(if any)*
Laboratory of Immunology, NIAID
Cetus Corporation

LAB/BRANCH
Surgical Neurology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NINCDS, National Institutes of Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Monoclonal antibodies selectively bind tumor cell differentiating antigens in vitro and in vivo. Natural effector mechanisms often do not mediate killing of monoclonal antibody bound cells so we have devised methods of linking extremely toxic proteins to the antibodies to selectively kill tumor cells.

Two methods of coupling toxic proteins, like ricin to antibodies, have been used to kill antigen positive cells in vitro. Ricin has two subunits, the A subunit blocks protein synthesis when in the cytosol and the B subunit binds galactose groups on all cell surfaces but also facilitates the transport of ricin A chain to the cytosol. 1) Linkage of the ricin A chain to antibodies yields reagents with low nontarget cell toxicity but target cell toxicity too slow for in vivo applications; 2) Linkage of intact ricin to antibodies results in very potent target cell toxicity but the nontarget cell killing must be prevented by a ligand which blocks ricin B chain binding to cells. This has limited to application to in vitro situations where 100 mM lactose can block ricin binding.

We are testing several new approaches to apply immunotoxins in vivo. 1) Cloning of toxins then altering their structure at the gene level to decrease nontarget cell toxicity; 2) Chemical modification of ricin to determine the location of the ricin galactose binding site and to possibly improve efficacy of ricin linked to antibodies; 3) Develop new ways to block the nontarget cell toxicity of ricin in vivo. We have discovered a monoclonal antibody which blocks the ricin galactose binding site similar to lactose. Preliminary in vivo trials in guinea pigs show over a 2 fold extension of survival time with intact ricin immunotoxins with no toxicity to animals. Anticancer trials of these new approaches are ongoing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	**PROJECT NUMBER** Z01 NS 02695-01 SN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Loss of Differentiation Function in Transformed Glial Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Joseph Bressler, Ph.D. Senior Staff Fellow

COOPERATING UNITS *(if any)*
Laboratory of Cellular Carcinogenesis and Tumor Promotion, LCCTP, DCE, NCI

LAB/BRANCH
Surgical Neurology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NINCDS, National Institutes of Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To obtain a better understanding of the relationship between differentiation and neoplasia our laboratory has been studying the effects of tumor promoting phorbol esters on glial differentiation. Previously we found that phorbol myristate acetate (PMA), the most potent tumor promoter, inhibits the glucocorticoid increase in glycerol phosphate dehydrogenase (GPDH) activity, which is an oligodengroglial specific property in the rat. The effectiveness of phorbol ester analogs to bind to the phorbol ester receptor, protein kinase C, correlates with their effectiveness in inhibiting GPDH induction. In the past year we have found that the natural ligand for the protein kinase C, diacylgylcerols, mimics the phorbol ester effect.

Tumor promotion can be divided into two stages. Stage 2 promoters were found to be more active than stage I promoters in inhibiting GPDH induction. Binding and degradation studies suggest that the increased activity of stage 1 promoters is either their rate of degradation or the presence of specific stage 2 receptor.

Another aspect of glial differentiation being studied is the regulation of intermediary filament expression. During development, astroglial derived cells switch from synthesizing vimentin to synthesizing glial fibrous acidic protein (GFAP). Glioma cells in culture consistently express vimentin and in a few cases coexpress vimentin with GFAP. We have not been able to alter the expression of filaments in glioma cells by incubation with differentiating agents. In addition, human glioma cell cultures synthesize vimentin and GFAP when grown as reaggregating cultures, or grown on polylysine or collagen. Primary monolayer cultures of astroglial cells synthesize vimentin and GFAP but synthesize only GFAP when grown as reaggregating cultures. The differences in vimentin synthesis between reggregating astroglial cell cultures and reaggregating human glioma cultures may reflect changes in regulatory mechanisms which occurred during transformation.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	**PROJECT NUMBER** Z01 NS 02696-01 SN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Intraarterial Chemotherapy Combined with Extracorporeal Drug Removal

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Edward H. Oldfield, M.D.	Principal Investigator
Dr. Robert Dedrick	Bioengineering and Instrumentation Branch Division of Research Services
John Doppman, M.D.	Diagnostic Radiology Department, CC

COOPERATING UNITS *(if any)*
Bioengineering and Instrumentation Branch, Division of Research Services
Diagnostic Radiology Department, Clinical Center

LAB/BRANCH
Surgical Neurology Branch

SECTION
Clinical Neurosurgery Section

INSTITUTE AND LOCATION
NINCDS, National Institutes of Health, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

One of the basic tenets of anticancer chemotherapy is that increased tumor exposure to drug should result in increased tumor response. One method currently being used to increase drug exposure of malignant brain tumors is by intracarotid infusion. Although intracarotid infusion increases drug delivery, systemic toxicity, not brain toxicity, frequently limits the tolerable dose. A means of extracting the drug from the blood after one passage through the brain, and before the high concentration of drug reaches the general circulation, should allow dose escalation to levels which provide increased tumor response. The following have been demonstrated.

1) A pilot study of 4 patients treated with intracarotid BCNU during extracorporeal hemoperfusion of the jugular venous blood demonstrated that the drug exposure of the body could be reduced by 56-87% by channeling the blood extracorporeally at 300 ml/min through a hemoperfusion cartridge for drug extraction. The pharmacokinetic advantage (ratio of brain exposure to body exposure) compared to intravenous infusion of the same dose was 21-55:1.

2) Drug streaming during intracarotid delivery results in maldistribution of the infused agent with the potential of delivering very high (toxic) concentrations of drug to some regions of the brain, while other areas receive minimal drug. Drug streaming is probably the cause of focal encephalomalacia following intracarotid infusion of BCNU.

3) A dye dilution study of intracarotid infusion of indocyanine green demonstrated that the recovery of substances from the jugular blood after intracarotid infusion is linearly related to the rate of aspiration of the blood from the jugular blood. This suggests that the amount of an injected drug which can be removed from blood before the blood is returned to the body is dependent on the rate at which blood is withdrawn from the jugular vein.

4) Extracorporeal circulation of the jugular blood for drug removal during intracarotid infusion of cis-platinum resulted in tumor exposures 5-15 flow greater than the exposure of the remainder for the body.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02454-05 SN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biological Studies of Human Pituitary Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Edward H. Oldfield, M.D.	Senior Investigator

COOPERATING UNITS *(if any)*
Developmental Endocrinology Branch, NINCDS
Diagnostic Radiology, CC

LAB/BRANCH
Surgical Neurology Branch

SECTION
Clinical Neurosurgery Section

INSTITUTE AND LOCATION
NINCDS, National Institutes of Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The influence of the hypothalamic releasing factors CRF and GRF on the hormonal secretion of pituitary adenomas has been determined in vitro and correlated with the patients' response in vivo. These studies indicate that the pituitary tumors causing Cushing's disease, Nelson's Syndrome and acromegaly are responsive to their appropriate releasing factor. We are investigating the potential of using the releasing factors conjugated to toxic proteins to effect cytotoxicity of pituitary tumors in vitro. The preliminary results are encouraging.

We have also investigated the use of venous sampling to aid in the diagnosis and treatment of patients with Cushing's syndrome. Our results, which now include 55 patients with Cushing's syndrome, suggest that the procedure will be of significant benefit in: 1) establishing the diagnosis of Cushing's disease and 2) determining preoperatively the site of pituitary microadenomas.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02697-01 SN

PERIOD COVERED

October 1, 1984 through September 31, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Evaluation of Metabolic Differential Between Brain and Brain Tumor with Thiopental

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Edward Oldfield, M.D.	Principal Investigator
J. Bob Blacklock, M.D.	Senior Staff Fellow

COOPERATING UNITS *(if any)*

Giovanni DiChiro, M.D., NIS, ODIR, NINCDS

LAB/BRANCH

Surgical Neurology Branch

SECTION

Clinical Neurosurgery Section

INSTITUTE AND LOCATION

NINCDS, National Institutes of Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project involves FDG-PET scanning of patients who have brain tumors before and during deep barbiturate general anesthesia. The results indicate that a profound reduction of cerebral glycolytic activity can be achieved with a level of anesthesia which produces burst-suppression EEG activity. Gliomas, however, have only a minimal change in glycolytic rate under the barbiturate anesthesia. The results also indicate that lower grade lesions, which are not visible on PET scans performed with the patient awake, become visible as background synaptic activity is suppressed with barbiturates. The true extent of growth into the surrounding tissue by higher grade lesions can be better appreciated when background activity is reduced under the barbiturate anesthesia. This work provides evidence that barbiturates may allow a "reverse contrast enhancement" of lesions with decreased neuronal activity. This phenomenon may provide a basis for development of specific antitumor therapy. The technique also may be valuable in studying other pathological processes such as degenerative diseases, epilepsy, movement disorders, cerebral infarction and head injury.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 NS 02367-07 SN

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Biological, Immunological and Chemotherapeutic Studies of Human Brain Tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Paul L. Kornblith, M.D.	Chief, Surgical Neurology Branch
Joseph Bressler, Ph.D.	Senior Staff Fellow
Elizabeth A. Grimm, Ph.D.	Senior Staff Fellow
Conrad Kufta, M.D.	Senior Staff Fellow
Steven Jacobs, M.D.	Senior Staff Fellow
Edward Oldfield, M.D.	Medical Officer
Donald Wright, M.D.	Medical Officer
Richard Youle, Ph.D.	Senior Investigator

COOPERATING UNITS (if any)
Radiation Oncology Branch, NCI; NCI-Navy Medical Oncology Branch; BEIB, DRS, NIH
DuPont Company, Biomedical Products Department, Glenolden, PA
Arizona State University, Cancer Research Institute, Tempe, AZ

LAB/BRANCH
Surgical Neurology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NINCDS, National Institutes of Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.0	6.0	1.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Human brain tumors are evaluated in a tissue culture environment as to their basic biological behavior, their response to chemotherapeutic agents and the detailed immunological interactions between the host and the tumor. A primary goal is to improve the therapy of patients by understanding the basic cellular biology of malignant human brain tumors.

SNB has continued the biological characterization program with the inclusion of flow cytometry, karyotyping, glial fibrillary acid protein, fibronectin, S-100 and Factor VIII assays, DNA repair, adrenergic and other receptor assays, ganglioside and glycoprotein assays, cloning techniques, in-depth neuropathological studies, and automatic image analysis; utilized both aqueous and surface chemotherapy assays to test several new potential antiglioma agents and initiated a prospective in vitro selection of clinical trials with these agents; carried out protocols with AZQ, spiromustine and platinum derivatives; defined the basis of cellular sensitivity or resistance to nitrosoureas; characterized the humoral cellular immunological response to gliomas; and carried out correlative cellular and PET scan glucose metabolic studies.

PHS 6040 (Rev. 1/84)

GPO 914-918

☆ GPO : 1986 O - 86 - 151-062